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A 14-Day Ground-Based Hypokinesia Study in Nonhuman Primates - A Compilation of Results

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PREFACE

The 14-day ground-based hypokinesia study with rhesus monkeys described in this volume was conducted to determine if a spaceflight of similar duration might affect bone remodeling and calcium homeostasis. Such a mission with two rhesus monkeys is being developed by Soviet scientists for a launch in 1982. United States experiments, including those described in this report, are being readied for inclusion in this mission as part of the Joint US/USSR Biological Satellite Program.

Cooperation between the US and USSR in the area of space biology and medicine began in 1971 with the signing of the US/USSR Science and Applications Agreements. A Joint Working Group for Space Biology and Medicine was established and met periodically to exchange information obtained during spaceflights and to discuss problems and topics of mutual scientific interest. In October of 1974, during the fifth meeting of the Joint Working Group, the Soviets offered to fly US experiments aboard an unmanned spacecraft scheduled for launch in 1975. Eleven US biological experiments were included in the 1975 biosatellite mission, Cosmos 782, and since that time a total of 21 additional US experiments were flown; 7 on Cosmos 936 in 1977, and 14 on Cosmos 1129 in 1979.

The experiments on these three biosatellites utilized rats, insects, and plant tissues to determine the effects of spaceflight on living systems. The flight with monkeys now in preparation is a unique opportunity to extend and expand the interesting results obtained with rats, to specimens more closely related to man. The results of the study described herein is a first step in preparing experiments to take advantage of this unique opportunity.

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A 14-DAY GROUND-BASED HYPOKINESIA STUDY IN NONHUMAN PRIMATES — A COMPILATION OF RESULTS

A 14-day primate hypokinesia study was undertaken to determine what changes in calcium metabolism and bone might be demonstrable following a spaceflight of approximately 2 weeks.

The subjects were 7 male rhesus (Macaca mulatta) ranging in weight from 6 to 10 kg. Two animals served as controls; the remaining 5 monkeys were wrapped in several layers of soft-roll cast padding; quick-setting plaster-of-Paris-impregnated bandages were used to construct a full body cast. Post-casting position approximated that seen in a relaxed primate in zero gravity, i.e., limb and trunk upright. The experimental monkeys were placed on an A-frame in an upright position for the 2-week casted period. The control monkeys were placed in metabolic cages.

The casted monkeys were hand fed three times per day; food was placed in the food tray of the controls. Food consisted of Purina Monkey Chow and fruit. Tap water was available ad libitum. Food and water consumption were monitored and recorded. A single Purina feedlot was used throughout the experiment. The monkeys were housed in an air-conditioned, windowless room, illuminated with fluorescent light 12 hr a day. The temperature of the room was 24 ±1°C.

The monkeys were initially surveyed radiographically to determine base-line data. Ten-ml blood samples were drawn on T-8, T+4, T+11, T+18, and T+23 days, with T being the date of casting. The venous blood was allowed to clot at ambient temperature for 3 ±1 hr and was then placed in a refrigerated centrifuge and spun at 2000 rpm for 30 min. Serum was drawn off, placed in glass vials, and frozen at -20°C until analyzed for calcium, phosphorus, total protein, parathyroid hormone (PTH), and vitamin D metabolites.

To determine bone formation parameters, 20 mg/kg oxytetracycline or declomycin was administered by I.V. infusion on T-10, T-1, T+12, and T+21 days; the labels were alternated starting with oxytetracycline. One control and two casted animals were sacrificed at T+13 days; the remaining casted monkeys were removed from the cast at T+14 and allowed to recover in standard cages until T+28 days when they were sacrificed. The control monkeys did not gain or lose weight during the experiment. The casted animals lost slightly less than 1 kg while in the casts.

Kazarian and Collins found marked changes in strength, energy to ultimate load, and bone stiffness of vertebrae from the casted animals (p. 4, this volume). Bone strength was only decreased 2% at the end of the casting period, but further decreased to 21% at the end of the 14-day recovery period. Displacement to ultimate load showed a 25% decrease at the end of casting, but only a 14% decrease at the end of the recovery period. Energy to ultimate load was decreased 39% at T+13 and showed a 46% decrease at T+28 days. Stiffness was 12% less at T+13 days and 44% depressed at T+28 days.

These data suggest that not only would vertebral strength parameters decrease during a 2-week flight, but also that such parameters would continue to decline for at least 2 weeks following flight.

Data on vertebral histomorphometry indicated that immobilization had a dramatic effect on trabecular bone which continued throughout the 14-day recovery period (Mathews et al., p. 16, this volume). Surface resorption increased dramatically and bone formation decreased drastically; formation surface was approximately 24% in the control animals, but only 3% in the casted group (T+13) which increased to 13% in the recovery period; total resorption was about 28% for control animals, and 46% for the casted monkeys at T+13 which only decreased to 36% at T+28. No osteoblasts were found during immobilization, and much of the available osteod had mineralized. The surface covered by osteoclasts increased 200 to 300% during immobilization but returned to normal levels after decasting. In summary, these data show a significant effect of 14 days of immobilization on the number and activity of bone cells, with a near complete cessation of bone formation and a 2- to 3-fold increase in the rate of bone destruction. A 14-day recovery period was not sufficient to reverse the changes.

Mathews et al. (p. 16, this volume) also obtained data on ulnar changes which showed significant impairment of cortical bone formation with two of the five immobilized animals exhibiting virtually complete cessation of bone formation toward the end of the casting period; in one monkey, bone formation resumed in the recovery period, although at a low rate. There was no significant difference in resorption spaces between casted and control animals. These data suggest that recovery in cortical bone may be more rapid than that in trabecular bone.

Serum minerals and hormones were analyzed by Cann and Arnaud (p. 29, this volume). The immobilized animals showed a progressive decrease in total calcium throughout the immobilization period with a large increase upon remobilization; values returned to normal by T+28. Calcium metabolic and endocrine measurements showed that changes do occur during short term immobilization.

It was noted by Simmons and Walker (p. 34, this volume) that immobilization reduced the labeling frequency in the osteons from the lingular and premolar regions of the mandible, with the bone being labeled largely during the control period. However, the rate of appositional bone growth did not appear retarded in those osteons which continued to grow during the experimental period. Osteon dimensions were unaffected by immobilization. Although most parameters remained unchanged in the mandible, the major effect of treatment was to retard the osteon birth or maturation rate. Russell and Simmons (p. 50, this volume) found no apparent change in Ca, P, or hydroxyproline (OHPr) content after analysis of either total mandibular tissue or that fraction of the mandible which is most mature (highest density fraction). However, they noted that concentrations of these moieties were lower than normal in the most recently formed bone and higher than normal in the maturing fraction of mandibular bone. These alterations were different from those noted in rats which had flown in space. Similar changes in the jaw would

have been surprising since the monkeys were in a sitting position and gravity gradients to the head were maintained.

The results of this experiment clearly show that very significant changes in calcium metabolism and bone turnover occur within a 2-week period of casting and continue throughout the 2-week recovery period. Although measurements of bone turnover, using tetracycline labeling, can be made even years after flight, definition of cell types, cell numbers, bone surfaces, and mineralization and/or matrix defects which are altered by flight would be impossible to determine unless biopsies or bone specimens were taken within several days of recovery. The in vivo tetracycline labeling technique is a very powerful tool for bone studies because sequential labels permit each animal to be used as its own control; thus, these data strongly suggest that valuable information on the effect of spaceflight on bone turnover could be obtained despite a small number of monkeys and a relatively short duration in orbit.

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STRENGTH CHARACTERISTICS OF THE ISOLATED VERTEBRAL CENTRUM

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INTRODUCTION

A 14-day ground-based nonhuman primate hypokinesia investigation was conducted to determine what changes, if any, in bone strength may be demonstrated following a spaceflight of approximately 2 weeks in duration. In this study, controlled compressive loads were applied to vertebral bodies excised from control and immobilized rhesus monkeys. The material properties reported herein were limited to ultimate load, displacement to ultimate load, stiffness, and energy to ultimate load. Other data including histological and radiographic were also obtained.

MATERIALS/METHODS

The Rhesus Monkeys

Seven clinically screened male rhesus monkeys (Macaca mulatta) ranging in weight from 6 to 10 kg were prepared for tests. Two animals served as controls, and five were casted. The controls are identified as A212 and A220, and the experimental animals as A396, 72B, A350, 48E, and A392. (A392 was an adult rhesus monkey, the remainder adolescents. For purposes of this investigation the results of A392 were not included because vertebral strength variations were found to be greater than expected.)

Sacrifice Schedule

At the end of approximately 2 weeks immobilization, A220 (control), and two immobilized animals (A396 and 72B) were killed. Their lower spinal column vertebrae (L1-L7) were mechanically tested. The remaining immobilized primates A350 and 48E were removed from their casts and placed in control cages. Primates A212 (control), A350, and 48E were killed 27 days following the start of the experiment. Their lower spinal vertebrae (L1-L7) were also removed and mechanically tested. The formal sacrifice schedule is shown in table 1 (R = recovery time in days).

Method of Testing Individual Vertebral Centra

The individual vertebral centra were disarticulated from one another by slicing through the midsection of the intervertebral disks, the articular capsules were sectioned, and the vertebral bodies individually segmented. The posterior aspects of the vertebral bodies were cut away. Each vertebral centrum was cleaned of all soft tissue clinging to its surface. The cartilaginous end plates were removed. Both the superior and inferior vertebral body-bearing surfaces were photographed. The photographs were used to collect vertebral body-bearing areas using a Keuffel-Esser Compensating Planimeter, and the results were averaged. The length of the vertebral body was measured using a Browne and Sharp Digital Vernier Caliper. To promote a uniform distribution of load, the bearing surface of each vertebral centra was potted in an acrylic compound. The potting compound produced circular-shaped pots with the specimen located centrally within. The specimens were now ready for testing. Each column position was compression tested using an MTS (Materials Testing System) testing machine. All specimens were tested at a loading rate of 0.889 m/s (2100 in./min). Following the compression tests the specimens were radiographed and some were prepared for histological studies.

The Load-Displacement Curve, Data Reduction Methodology

A typical test curve is shown in figure 1. The test specimen load is plotted on the ordinate versus the ram displacement (specimen deformation) on the abscissa. Specimens were tested at 50% strain, which is a maximum displacement equal to 50% of the original pretest height.

The first part on the load-displacement curve following the apparent elastic section, where the tangent to the curve becomes parallel to the displacement axis, is defined as the ultimate load. The deformation at this point is defined as the displacement to ultimate load. The ultimate load can also be looked at as the point at which the specimen has become permanently deformed and/or has been structurally damaged. The specimen stiffness (loading slope) is determined by fitting the apparent linear elastic section of the load-displacement curve with a least squares linear fit.

The energy to ultimate load is defined as the area under the load versus displacement curve, from the point of zero displacement up to the displacement at ultimate load. The ultimate engineering stress is computed by dividing the ultimate load by the specimen area. The strain at ultimate is computed by dividing the displacement at ultimate by the original specimen pretest height. The dependent variables that can be extracted from the load-displacement curve are summarized in table 2.

RESULTS

This report deals only with analysis of four dependent parameters of the load-displacement curve: (1) ultimate load, (2) displacement to ultimate

load, (3) energy to ultimate load, and (4) stiffness. A statistical analysis of variance was conducted on each material parameter measurement. The sources of variations considered were environment (control versus immobilized) and differences among subjects. The immobilized case was further broken down into two recovery levels, R+0 (no recovery) and R+15 (15-day recovery).

To simplify the analysis of variance calculations, column position (L1-L7) was not considered. From past rhesus compressive loading tests it was statistically shown that there is a significant column position dependence on all parameters except for, in some cases, the stiffness parameter. The statistical values presented represent the level of confidence of rejecting the hypothesis of no effect due to immobilization. Levels of significance greater than 0.95 were not considered. Levels of confidence as low as 0.60 were reported, since consistent trends were noted in the data and the large degree of scatter, due to the small number of test primates available, could prevent the trends from being statistically significant at a higher level of confidence.

Ultimate Load

Ultimate load versus vertebral level for control, R+0, and R+15 is shown in figure 2. The ultimate loads observed along with the analysis of variance table for these data are shown in table 3. The effect of immobilization was significant at the 90% level of confidence. The ultimate load increases as vertebral level increases (L1-L7). The ultimate load of R+15 showed a leveling off of load in the L5-L7 range. Ultimate load was greatest in the control and lowest in the R+15. No marked differences were noted between the control and the R+0. The difference in the control and the R+15 indicates that following immobilization and a short recovery period, the bone strength decreased approximately 20%. Since little difference can be seen between the R+0 and control curves, this could indicate that the effect of immobilization is not immediately apparent until normal control activity is resumed. Extended recovery time could possibly result in an increase in bone strength. Further investigation is necessary.

Displacement to Ultimate Load

Displacement to ultimate load versus vertebral level for control, R+0, and R+15 is shown in figure 3. The displacements observed along with the analysis of variance table for these data are shown in table 4. The effect of immobilization was not conclusive since it was significant only at the 60% level of confidence. The displacement to ultimate load increased with vertebral level (L1-L7). Although not conclusive, the plot indicates that displacement to ultimate load was less for the immobilized primates, a factor which correlates with the ultimate load plots. This indicates that less displacement and less force were required to fail the immobilized vertebrae, implying again that immobilization resulted in a decrease in bone strength. Information relating to the effects of recovery time was inconclusive. The

low level of confidence could be attributed to the small sample size (2 primates per group) available for experimentation.

Energy to Ultimate Load

Energy to ultimate load versus vertebral level for control, R+0, and R+15 is shown in figure 4. The energy values observed along with the analysis of variance table for these data are shown in table 5. The effect of immobilization was not totally conclusive since it was significant only at the 75% level of confidence. The energy to ultimate load increased with vertebral level (L1-L7). Energy to ultimate load was less for the immobilized groups when compared to the control group. Little difference was noted between the R+0 and R+15 immobilized test groups. This graph points to a trend that less energy was required to fail the immobilized vertebrae, indicating a possible loss of bone strength in the immobilized cases. As mentioned before, the low level of confidence can most probably be atrributed to the small sample size of primates available for testing.

Stiffness

Stiffness versus vertebral level for control, R+C, and R+15 is shown in figure 5. The stiffness values observed along with the analysis of variance table are shown in table 6. The effect of immobilization was significant at the 90% level of confidence. No significant effect due to vertebral level was apparent. Stiffness was greatest in the control and least in the R+15. No marked differences were apparent between the control and the R+0 groups. This plot further substantiates an apparent trend that immobilization results in some form of change in the bone structure, resulting in less bone strength and the bone being less stiff (resistance to load) than the control group counterparts. As was shown in the ultimate load plots, the R+15 group was significantly lower than the R+0. Further investigation is necessary.

CONCLUSIONS

The results of this hypokinesia study indicate, but not conclusively, that rhesus monkeys exposed to 2 weeks of immobilization exhibit a reduction or loss of bone strength. It was estimated from the experimental data that this reduction in strength was on the order of 20%. In general, no marked difference in bone strength was apparent immediately after immobilization (R+0), but loss of bone strength was statistically significant in the 15-day recovery group (R+15). This could indicate that the effect of immobilization is not immediately apparent until normal control activity is resumed. It is also suggested that an extended recovery time could result in a real recovery, resulting in an increase in bone strength approaching the control group.

Based on the results of this study, it is suggested that further experiments be conducted to fully clarify the effects of immobilization. Further

studies should include a much larger primate sample size as well as a more detailed statistical analysis of all material properties. Also, future experiments should include a test group exposed to a much longer recovery period.

TABLE 1.- SACRIFICE SCHEDULE

Group	Rhesus number	Sacrifice schedule
Control	1 - Rhesus A220	R+0
	2 - Rhesus A212	R+15
Immobilized	1 - Rhesus A396	R+0
	2 - Rhesus 72B	R+0
	3 - Rhesus A350	R+15
	4 - Rhesus 48E	R+15

TABLE 2.- MATERIAL PARAMETERS OBTAINED FROM FORCE VERSUS DISPLACEMENT CURVES

1. Stiffness - N/m - initial linear portion of loading curve
2. Ultimate Load - N - portion of curve where the tangent to the curve becomes parallel to the displacement axis (failure load)
3. Displacement to Ultimate Load - m - corresponding displacement value at ultimate load on F vs D curve
4. Ultimate Engineering Stress - PASCALS - $\frac{\text{Ultimate Load}}{\text{Pretest Area}}$
5. Ultimate Engineering Strain - m/m - $\frac{\text{Displacement at Ultimate Load}}{\text{Pretest Height}}$
6. Energy to Ultimate Load - JOULES - area under loading curve from zero displacement up to displacement at ultimate

TABLE 3.- IMMOBILIZATION STUDY - DATA
SUMMARY AT 0.889 m/s
ULTIMATE LOAD - N

Cp	Control	R+0	R+15
L1	5.0940E+05	5.2175E+03	3.6815E+03
L2	5.2605E+03	5.5135E+03	4.3200E+03
L3	5.6315E+03	4.6675E+03	4.5225E+03
L4	5.6905E+03	5.7660E+03	5.8560E+03
L5	6.0175E+03	6.3945E+03	4.6610E+03
L6	6.3005E+03	6.4615E+03	4.6615E+03
L7	6.7145E+03	6.0400E+03	4.5750E+03

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	F ratio	Level of confidence
Environment (Control/immobilized)	2	55.520	7.107	0.90
Error (Subject/environment interactions)	3	7.811		

TABLE 4.- IMMOBILIZATION STUDY - DATA
SUMMARY AT 0.889 m/s
DISPLACEMENT TO ULTIMATE LOAD - m

Cp	Control	R+0	R+15
L1	3.3400E-03	2.0430E-03	2.3045E-03
L2	2.8690E-03	2.4765E-03	3.0705E-03
L3	3.2290E-03	1.9085E-03	3.0915E-03
L4	3.4820E-03	2.4780E-03	3.6845E-03
L5	3.8370E-03	2.9900E-03	2.9710E-03
L6	4.6045E-03	3.3710E-03	3.7465E-03
L7	4.7120E-03	4.3850E-03	3.6085E-03

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	F ratio	Level of confidence
Environment (Control/immobilized)	2	21.339	1.447	0.60
Error (Subject/environment interactions)	3	14.743		

TABLE 5.- IMMOBILIZATION STUDY - DATA
SUMMARY AT 0.889 m/s
ENERGY TO ULTIMATE - JOULES

Cp	Control	R+0	R+15
L1	12.9600E+00	7.6035E+00	5.8740E+00
L2	11.7050E+00	10.2800E+00	10.0750E+00
L3	14.6350E+00	5.7845E+00	10.0415E+00
L4	16.6200E+00	9.2875E+00	13.0600E+00
L5	19.2350E+00	14.5295E+00	10.3195E+00
L6	22.9050E+00	16.7350E+00	12.9250E+00
L7	20.1000E+00	19.0150E+00	11.9900E+00

ANALYSIS OF VARIANCE

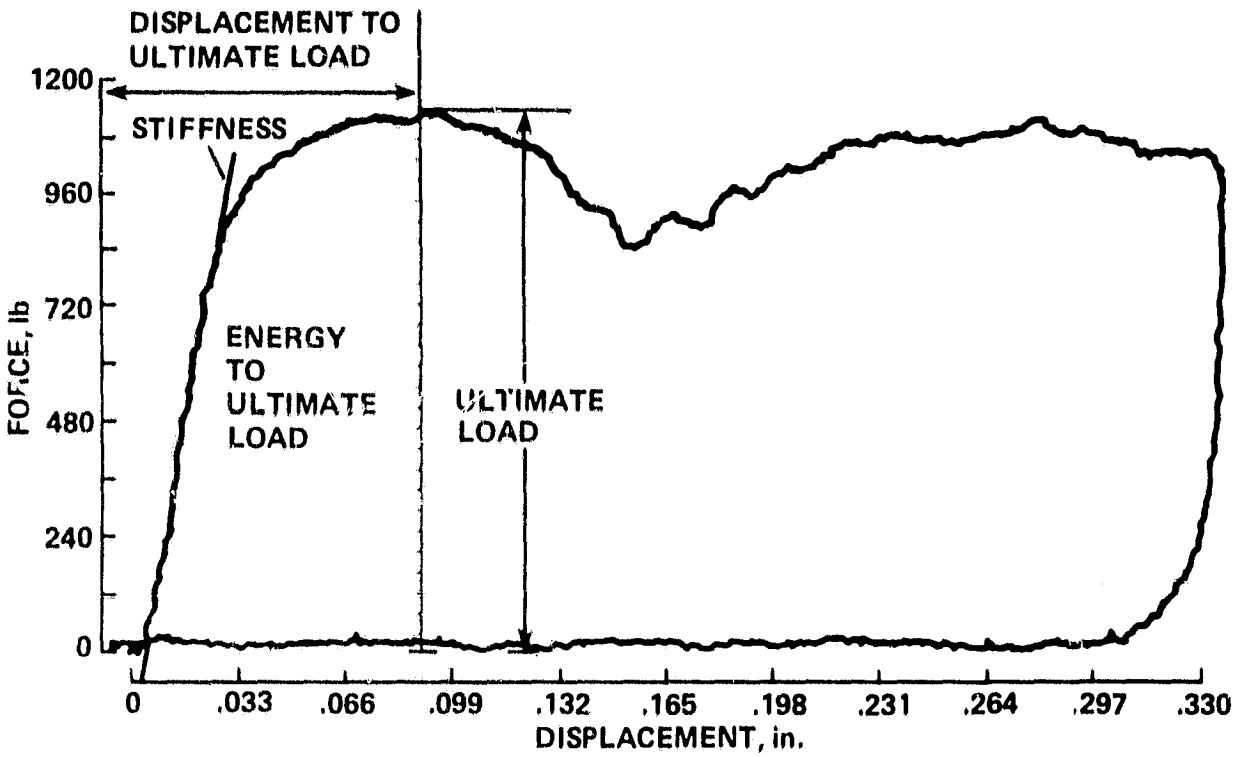
Source of variation	Degrees of freedom	Mean square	F ratio	Level of confidence
Environment (Control/immobilized)	2	1074.817	2.434	0.75
Error (Subject/environment interactions)	3	441.524		

TABLE 6.- IMMOBILIZATION STUDY - DATA
SUMMARY AT 0.889 m/s
STIFFNESS - N/m

Cp	Control	R+0	R+15
L1	3.5250E+05	3.7895E+05	1.9170E+05
L2	3.9110E+05	3.8390E+05	2.7435E+05
L3	4.4730E+05	3.8205E+05	2.2495E+05
L4	4.9975E+05	2.9340E+05	1.5985E+05
L5	4.6040E+05	3.4705E+05	2.2140E+05
L6	3.6020E+05	3.9745E+05	2.2815E+05
L7	3.5550E+05	3.4160E+05	2.5435E+05

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	F ratio	Level of confidence
Environment (Control/immobilized)	2	92.626	6.158	0.90
Error (Subject/environment interactions)	3	15.041		



TEST NUMBER	= 406
SUBJECT ID	= RHESUS 72B
COLUMN POSITION	= 5
VB LEVEL	= L2
DISPLACEMENT RATE	= 2100 in./min OR 0.889 m/s
MAXIMUM STRAIN	= 0.5000 in./min
SPECIMEN PRETEST LENGTH	= 0.6600 in.
SPECIMEN PRETEST AVERAGE AREA	= 0.3710 sq. in.
LOAD CAL	= 2500 lb
DISPLACEMENT CAL	= 0.5000 in.

Figure 1.- Typical monkey vertebral body compression load — displacement curve.

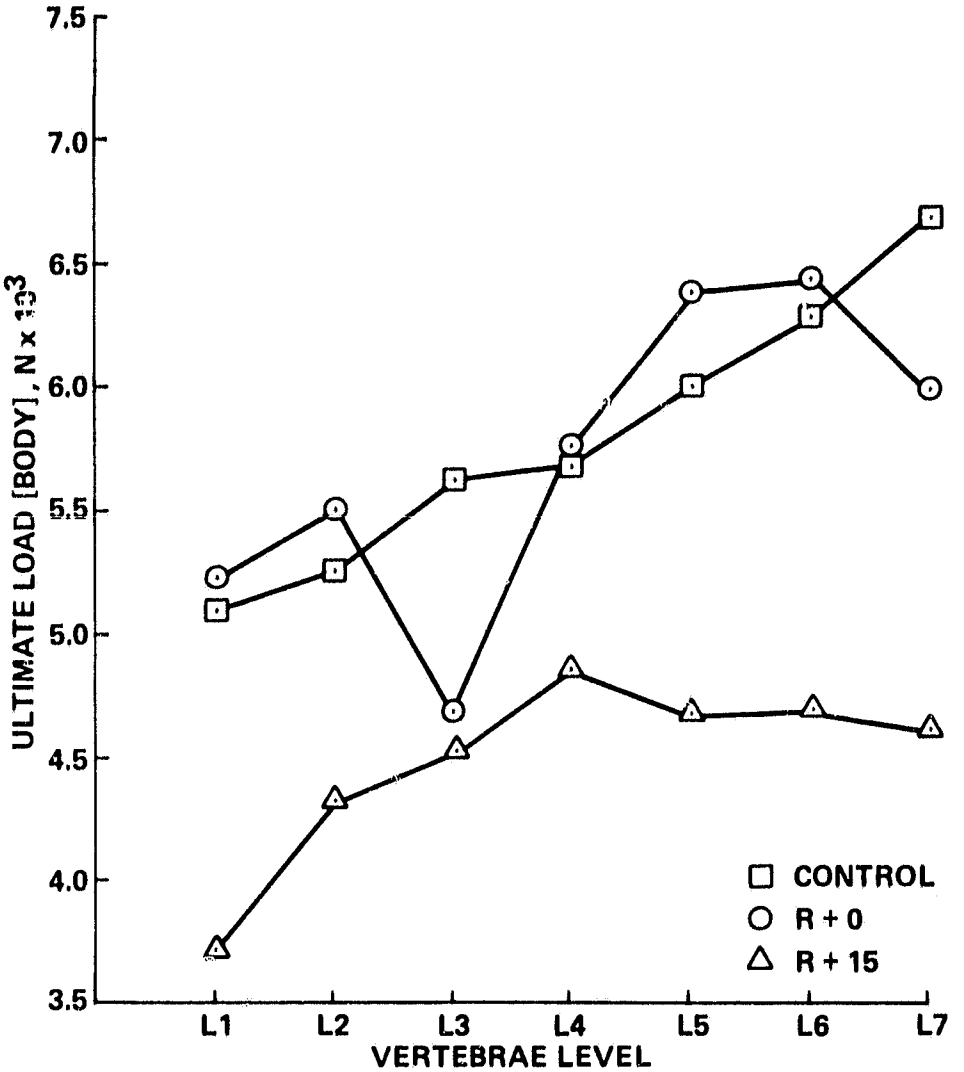


Figure 2.- Rhesus immobilized study — control, R+0 and R+15 at 0.889 m/s.

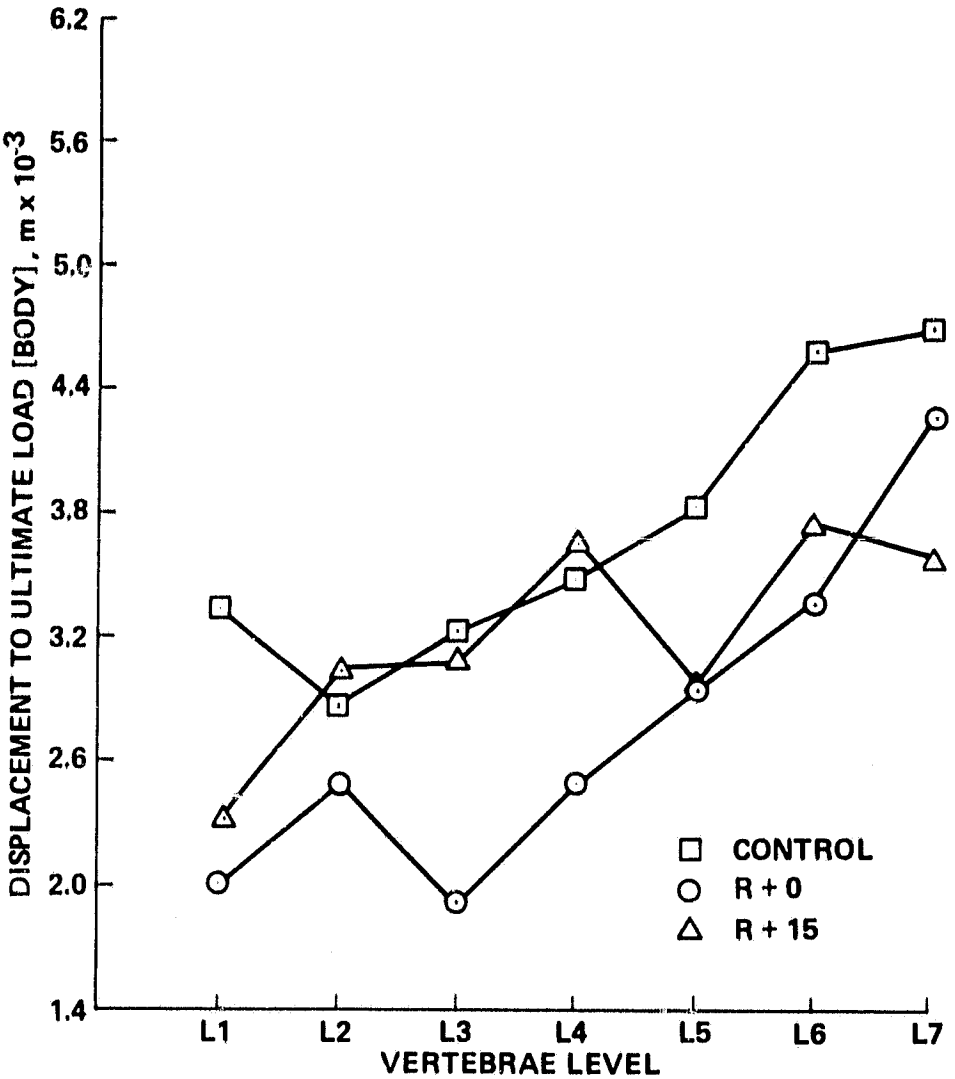


Figure 3.- Rhesus immobilized study — control, R+0 and R+15 at 0.889 m/s.

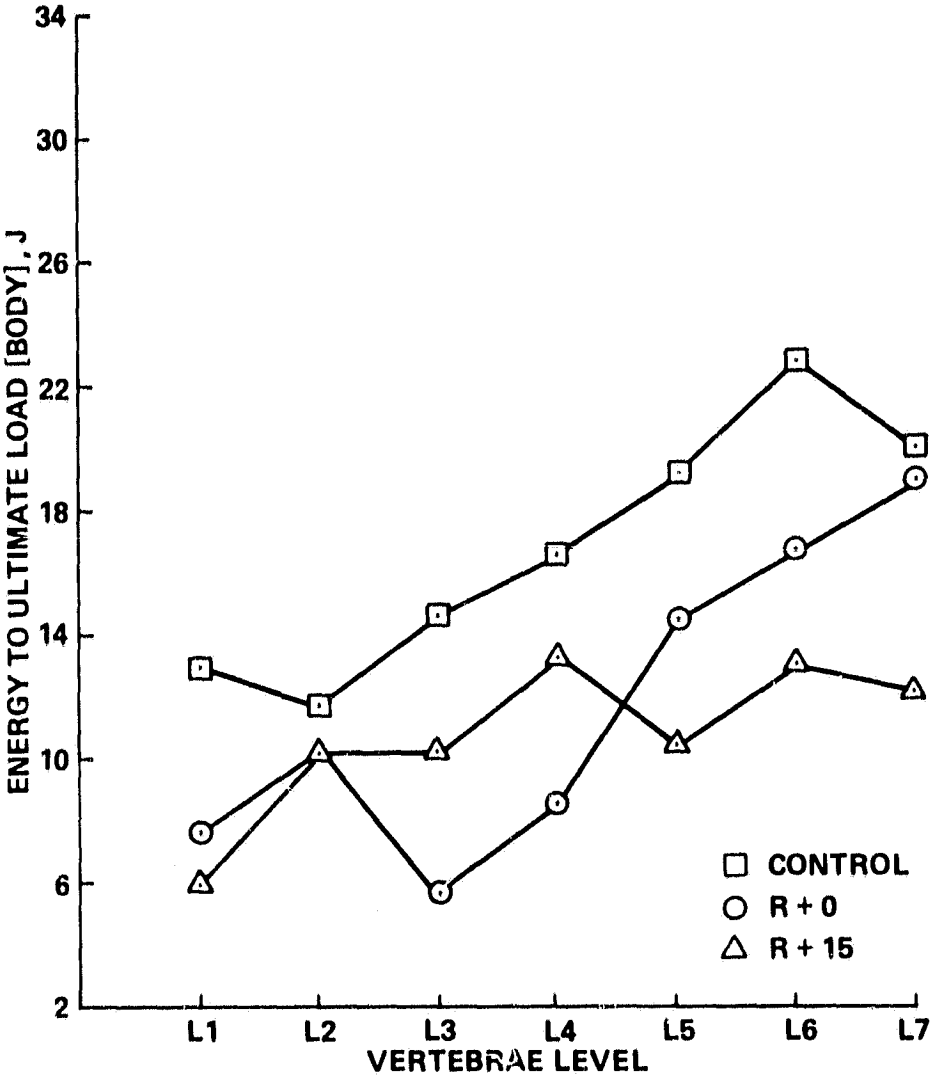


Figure 4.- Rhesus immobilized study — control, R+0 and R+15 at 0.889 m/s.

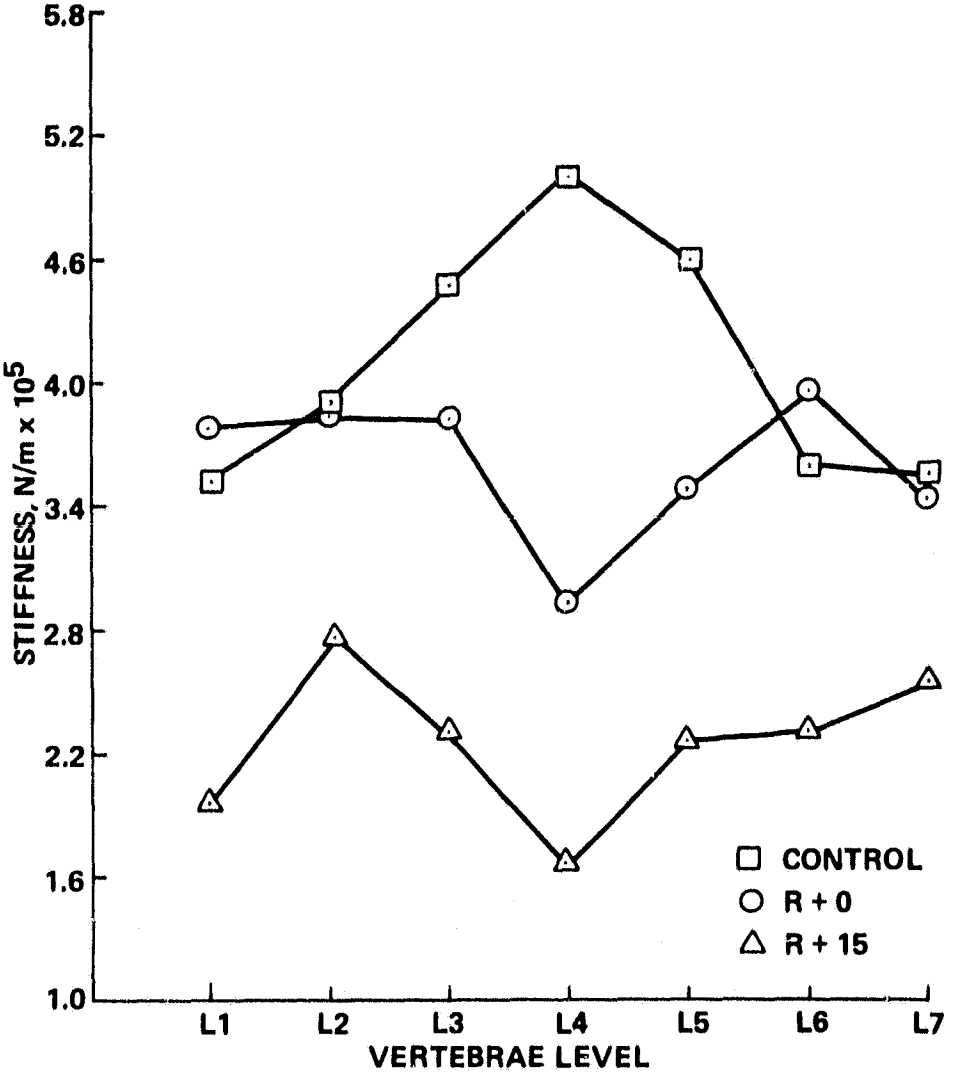


Figure 5.- Rhesus immobilized study — control, R+0 and R+15 at 0.889 m/s.

HYPOGRAVITATIONAL EFFECTS OF HYPODYNAMIA ON BONE CELL FUNCTION AND THE DYNAMICS OF BONE REMODELING

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PART 1: ANALYSIS OF VERTEBRAE

Data in this report have been obtained from the trabecular bone tissue of the vertebrae. Emphasis is on the cellular elements and their relation to resorbing and forming surfaces. The mineralization process as observed by double tetracycline labeling was analyzed in detail on cortical bone in the ulna (part 2, page 19).

METHODS

One complete vertebral body (T5, T6, or T7) was dissected from each animal, fixed in 70% ethanol, stained for 2 to 3 days in the Villanueva Bone Stain, dehydrated, infiltrated in methyl methacrylate monomer, and embedded in partially polymerized polymethyl methacrylate. Curing was done under vacuum at 37°C. Sections were cut in a vertical direction, i.e., from disc to disc, on a Jung-K microtome at a thickness of 5, 10, and 25 μm. These sections were mounted without further staining and used to do all the histomorphometric analyses. This was possible due to the prestaining method, which provides a section with very clear cellular and osteoid detail (figs. 1 and 3). In addition, due to the neutral pH of the stain, tetracycline labels can be seen very clearly on these sections (figs. 2 and 4). Special stains such as Goldner's Trichome (as modified by Villanueva) and Toluidine Blue were done on additional sections cut at 5 μm.

All measurements were made on a Zeiss microscope with a digitizing tablet connected to an MOP microprocessor. The status of the animals (whether control or immobilized) was not known until all measurements were completed. A total area of tissue of approximately 10 mm^2 was analyzed on each animal; the total surface of bone measured averaged 37 mm. Measurements performed on each section included total area of marrow space, mineralized bone, and unmineralized bone (osteoid). The surfaces were classified according to 14 categories: 8 formation, 4 resorption, and 2 quiescent. Forming surfaces were first classified according to the presence or absence of tetracycline label and then further subdivided according to the type of cells lining the osteoid, i.e., preosteoblasts, cuboidal or flat osteoblasts, and other,

unclassifiable cells. Resorbing surfaces were classified according to the presence or absence of osteoclasts within their scalloped, crenated domain. Also noted was the presence or absence of tetracycline labeling at the site of resorption. Quiescent surfaces were subdivided according to the presence or absence of previously laid down tetracycline.

Osteoid seam widths were measured directly. The average value for each section was multiplied by the total length of osteoid surface to obtain the osteoid volume.

RESULTS

The animals have been subdivided into three groups. Animals A212 and A220 in the Control (C) group, animals 72B, A392, and A396 in the Immediate Sacrifice (IS) group, that is, those sacrificed immediately after decasting, and animals 48E and A350 in the "Reconditioning" (RC) group, that is, those allowed to live for 2 weeks after decasting, before being sacrificed.

Table 1 shows the data of the three major categories of bone surfaces, that is, forming, resorbing, and quiescent surfaces. Also shown is the sum of resorbing and quiescent surfaces, that is, nonosteoid surface. The values are all expressed as a percentage of total surface (TS). Formation surface was approximately 24% in the control animals but only 3% in the IS group, increasing to 13% in the animals that were allowed to recover for 2 weeks. The extent of total resorption is about 28% for control animals, 46% for the IS animals, and 36% for the RC group. Thus, this shows a large increase in resorption surface. The extent of quiescent surface varies from 37% to 63%, and averages about the same for all three groups.

Table 2 shows the surface extent of osteoblasts (OBL) and osteoclasts (OCL). Osteoblasts are plump, basophilic cells of cuboidal or oblong shape. Osteoclasts are large multinucleated cells considered to be actively resorbing bone tissue (fig. 3). Values are expressed as percentage of total tissue (%TS), and of osteoid surface (%OS) or resorptive surface (%RS), respectively. The results show that osteoblasts completely disappear during immobilization. During reconditioning, one animal (48E) showed continued absence of osteoblasts; in the other animal (A350), some osteoblasts partly returned.

The extent of surface covered by osteoclasts showed a 2- to 3-fold (200-300%) increase during immobilization, returning to normal levels after decasting.

Measurement of the width of osteoid seams is shown in Table 3. The average value for the controls is 8.5 μm, for the IS group 3.1 μm, and for the RC group 8.5 μm. The relative osteoid volume [osteoid expressed as a percentage of total bone tissue (%TBT)] shows similar results: Controls 2.5%, IS 0.12%, RC 2.0%. These results would be expected with osteoblastic activity being depressed during immobilization and apparently recovering during the reconditioning period. Values for total bone volume (TBV) and mean trabecular

thickness (MTT) are also shown in this table, but as these values vary a great deal from site to site, the changes may not be significant.

When tetracycline is administered to an animal, it is laid down in the bone in areas of active mineralization and is thus an indirect indicator of sites of osteoid formation and, therefore, of newly formed bone. In this experiment, all the animals were labeled with tetracycline 10 days and 1 day before immobilization, and those that were allowed to recondition were labeled 2 days before and 7 days after decasting. In control A220 and all the animals sacrificed immediately post-immobilization (72, 392, and 396), the extent of tetracycline surface indicates the extent of surface which was forming bone during the pre-immobilization period. In areas where formation of osteoid ceased during the immobilization period but mineralization continued to completion, labeled quiescent surface (QS) is seen. The results (table 4) show an average of 40% TS with tetracycline label for these four animals. In the control animal, 16% TS is labeled quiescent surface, whereas in the immobilized animals, 35% is labeled quiescent surface. This means that in the control animal, 40% (16/41) of its osteoid-forming surface completed mineralization during the experimental period (approximately 2 weeks), whereas in the immobilized animals, 88% (35/40) of their previously osteoid-forming surface mineralized during the time of immobilization. This resulted, however, from suppression of new matrix synthesis, not from enhancement of mineralization.

In the animals that received four tetracycline labels (control 212 and RC 48 and 350), the extent of surface covered by the third and fourth labels (i.e., post-experimental) was measured (table 5). The control animal shows 10% TS with both third and fourth labels, whereas both reconditioned animals show less than 1% TS with both labels. Expressed differently, the control shows 95% of post-immobilization labeled surface with both labels, and the experimental animals show 7.7% of post-immobilization labeled surface with both bands of tetracycline. The extent of surface covered by only one of the post-immobilization labels was 0.5% TS for control, and 8.4% TS for the experimental animals.

DISCUSSION

Immobilization has a severe effect on trabecular bone: the surface extent, and by inference, the rate of resorption, increases drastically, and the rate of bone formation decreases drastically. An observation which is difficult to measure, but very obvious when looking at the histologic sections, is the depth of the resorption cavities. It appears that not only is there an increase in the number of osteoclasts, but there is also an increase in their activity. The photomicrographs indicate just two of these sites where deep resorption is occurring. Note also in these figures that the bone being resorbed is very new bone; it was laid down during the 10-day period immediately before immobilization, as indicated by the 2 tetracycline bands (these animals were in the IS group and thus only received pre-experimental labels).

There is much evidence in our data for severe curtailment of bone formation during the immobilization period. With the absence of osteoblasts, no new osteoid is being formed, as seen in the greatly reduced extent of surface covered by osteoid as well as the decrease in width of the osteoid. Mineralization continues after the onset of immobilization, as is seen by the extent of labeled quiescent surface. The animals that are allowed to recover show very little third label, probably because very little osteoid was available for mineralization at the time of decasting. During the recovery time, osteoblastic activity resumes producing new osteoid, which is then available for mineralization during the last labeling episode. If this is true, our data indicate that osteoblasts do not directly control mineralization. A recovery time of 2 weeks is evidently not sufficient for bone cell activity to return to normal levels; a period of 4 to 8 weeks would be more desirable.

In summary, our data indicate a large effect of a short period of immobilization on the number and activity of bone cells, with a near complete cessation of bone formation, and a 2- to 3-fold increase in the rate of bone destruction. If these changes continued during long-term immobilization, severe loss of bone would inevitably occur.

PART 2: ANALYSIS OF THE ULNA

Sections from all bones, that is, rib, ulna, radius, humerus, tibia, fibula, and femur, were scanned to determine the most suitable bone for initial measurements. It appeared that the greatest amount of tetracycline fluorescence was present in sections from the ulna. To give the greatest chance of demonstrating a significant difference, this bone was selected for study.

METHODS

The bones had been fixed in 70% ethanol and frozen. For shipment, they had been wrapped in ethanol-soaked gauze and placed in zip-lock bags; they arrived on April 8. After further trimming and cleaning, thick sections (300 μm) were cut from the sixth rib, ulna, radius, humerus, tibia, fibula, and femur with a Bronwill microtome. These were hand ground to 75- to 100-μm thickness with carborundum paper under running water. From each bone, sections were stained with the Villanueva Bone Stain for either 1 hr, 24 hr, or 48 hr, differentiated with 0.01% acetic acid and 95% methanol, dehydrated in 95% followed by 100% ethanol, and mounted with Eukitt medium. Additional sections were unstained and dehydrated, cleared, and mounted in the same way. Segments of the sixth rib and some whole vertebrae were embedded in polymethyl methacrylate from which thin (5-7 μm) sections will be cut using the Jung microtome. All measurements were made with a digitizing tablet connected to an MOP dedicated computer. The status of the animals, whether control or immobilized, was not known until the measurements were completed.

On the stained sections we measured total cortical area, total endosteal and periosteal perimeter, osteoid seam width, and the number of osteoid seams and resorption spaces. On the unstained sections, each labeled osteon with a seam was classified according to its labeling pattern to indicate in which time period bone formation began at that site (see appendix). The labeling sequence permitted unequivocal classification, except when only two labels of sequence O-D were present. In these cases, the location of the labels with respect to the stage of radial closure resolved any ambiguity. The total number of each of the four labels was counted for each section. The distance between adjacent labels was measured at four to six approximately equally spaced locations in each osteon. When more than two labels were present, the distances between each adjacent pair of labels were measured separately. The results were tabulated for each osteon and for each labeling period.

RESULTS

The static data are shown in table 6. There was no significant difference between control and experimental animals, except for a larger number of osteoid seams and resorption spaces in the immobilized animals of borderline statistical significance.

The assignment of individual labeled osteons to birth during different time periods is shown in table 7. There was no significant difference in the numbers of seams started at different time periods between control and immobilized animals, except for approximately twice as many seams in the immobilized animals which showed the first label and therefore were already present before the start of the experiment. This indicates that the difference found in the static measurement is a consequence of random variation in bone turnover between different animals and is not a result of the experimental procedure.

The numbers of individual labels are shown in table 8. There was a significant deficit of third labels in the immobilized animals. This deficit was not evenly divided, but resulted from almost complete absence of the third label in two of the five animals. In one of these, where the sacrifice was delayed, the fourth label was present in every osteon in which the first or second labels were present, indicating that the arrest of bone formation was temporary and that bone formation resumed in the post-immobilization period. The other animal with absence of the third label was sacrificed before the fourth label was given.

The results of the appositional rate measurements at different time periods are shown in tables 9 and 10. The mean value fell slightly in the second and third time periods in the control animals, but the change from the control value was significantly greater in the immobilized animals. In the animal in which the fourth label was present but not the third, the apposition rate was measured between the second and fourth labels, and the values were included in the means for both the second and third periods.

DISCUSSION

Because of the time constraints of the pilot experiment, the measurements were limited, both in type and in number. Nevertheless, the data show a significant impairment of cortical bone formation produced by a short period of immobilization in a whole-body cast. Two of the five immobilized animals had virtually complete cessation of bone formation toward the end of the casting period, at the time of the third label; in one of these, formation resumed in the post-immobilization recovery period, although at a lower rate. The pooled data for all animals showed a modest but significant reduction in mineral apposition rate in the immobilized animals.

A possible fallacy in the interpretation of the appositional rate measurements must be considered. It has been shown that the appositional rate is fastest at the onset of bone formation in a recently complete resorption space, and falls progressively as the cavity is filled in concentrically toward the future Haversian canal. Consequently, in osteons showing all four labels, the three serial apposition rate measurements will inevitably show a progressive fall, even though the average for the whole bone remains unchanged. In a steady state, the high apposition rate measured between the first two labels in seams active throughout the period will be balanced by a low rate in seams which are nearing completion and therefore show only the first and second labels. Conversely, the low appositional rate measured between the last two labels in the seams active throughout the experiment will be balanced by a high rate in seams recently initiated and therefore showing only the third and fourth labels.

In the present experiment, with a very limited sample from a small number of animals, it would be expected that random variation in the age distribution of forming osteons could bias the mean apposition rate measurement. Preliminary inspection of the sections measured suggests that this bias was in fact somewhat greater in the control animals, and probably accounted partly for the apparent fall in the second and third periods. Furthermore, the large number of individual measurements makes it unlikely that a differential bias in the age distribution of individual osteoid seams could account for the observed difference in appositional rate.

Rigorous exclusion of this possible source of error will require measurements of cement line diameter, wall thickness, and Haversian canal diameter for each individual osteon which is labeled. From these measurements can be computed a value for percent radial closure. In normal dogs and cats, when apposition rate is plotted against percent radial closure on semilogarithmic paper, the values fall on a straight line. The regression of apposition rate against percent radial closure could be determined separately in the control and experimental animals, and the parameters of the regression equations tested for significant differences.

The findings of almost total suppression of bone formation in some bones at the end of the immobilization period, with resumption of bone formation during the recovery period, together with a fall in apposition rate, are remarkably similar to the observations of Moray and Baylink on periosteal bone formation in rats on a previous COSMOS flight. The results of the present study strongly suggest that valuable information on the effect of spaceflight on bone formation could be obtained, despite a small number of animals and a short duration of orbit. The in vivo tetracycline labeling technique and the use of sequential labels permit each animal to be used as its own control for appositional rate measurements. Furthermore, we have demonstrated the feasibility of using a simple sequence of labels involving only two different colors to classify forming osteons according to the onset of bone formation in different periods of the experiment.

SUMMARY

A short period of immobilization produced significant impairment of cortical bone formation despite a small number of animals. The use of multiple sequential labels, permitting each animal to act as its own control for appositional rate measurements, should prove to be a useful technique for studying the effect of spaceflight on primate bone.

APPENDIX

Osteons with an osteoid seam and the first label must have started forming bone before the experimental period (pre-1). Osteoid seams with the second label but not the first must have started forming bone during the pre-immobilization period. Osteoid seams with the third label, but not the first or second, must have started forming bone during the period of immobilization. Osteons with only the fourth label present must have started forming bone during the post-immobilization period, and finally, osteoid seams with no labels must have started forming bone in the week prior to sacrifice.

TABLE 1.- BONE SURFACE AS PERCENT OF TOTAL SURFACE (TS)

Type bone surface, %TS	Control		Immediate sacrifice			Reconditioned	
	A212	A220	72B	A392	A396	48E	A350
Forming (%TS)	24.4	23.9	1.5	5.8	2.7	17.5	9.1
Resorbing (%TS)	23.9	31.9	35.1	57.1	46.6	20.0	51.0
Quiescent (%TS)	51.7	44.1	63.3	37.1	50.7	62.5	40.0
Nonosteoid (%TS)	75.6	76.0	98.4	94.1	96.3	82.5	91.0

TABLE 2.- BONE CELL POPULATIONS

Bone cell type, %TS	Control		Immediate sacrifice			Reconditioned	
	A212	A220	72B	A392	A396	48E	A350
OBL (%TS)	3.6	4.2	0	0	0	0	0.8
OBL (%OS)	14.9	17.4	0	0	0	0	9.1
OCL (%TS)	.9	1.3	2.7	3.6	4.0	.3	2.0
OCL (%RS)	3.8	4.0	7.8	6.4	8.6	1.4	3.9

TABLE 3.- BONE PARAMETERS

Bone parameter[a]	Control		Immediate sacrifice			Reconditioned	
	212	220	72	392	396	48	350
MSW (μm)	9.0	8.1	3.4	2.7	3.2	8.5	8.6
ROV (%TBT)	2.7	2.4	.07	.2	.1	2.4	16
TBV (%TT)	26	27	30	30	32	26	21
MTT (μm)	9(	105	101	119	113	79	69

[a]Refer to text for parameter definitions.

TABLE 4.- LABELED BONE SURFACE

Surface parameter	Control	Immediate sacrifice		
	220	72	392	396
Total labeled surface (%TS)	41	46	40	33
Labeled quiescent surface (%TS)	16	43	34	29
Fractional quiescent label (%)	40	93	82	88

TABLE 5.- POST-IMMOBILIZATION LABELS

Label parameter	Control	Reconditioned	
	212	48	350
Total PILE[a] (%TS)	10	0.4	0.7
Single label PILE (%TS)	.5	11.8	4.9
Fractional double label of PILE (%)	95	3.5	12

[a]Post-Immobilization Labeling Episode (PILE)

TABLE 6.- COMPARISON OF STATIC MEASUREMENTS IN ULNA

Measurement	Control (2)	Immobilized (5)	Significance
Cortical area (mm^2)	32.76 ±1.51	32.61 ±6.77	NS
Endosteal perimeter (mm)	8.88 ±.72	8.70 ±2.07	NS
Periosteal perimeter (mm)	23.73 ±.78	22.77 ±2.60	NS
Osteoid seam width (μm)	7.77 ±.18	8.26 ±1.19	NS
No. of seams ($/mm^2$)	.255 ±.053	.678 ±.256	2.1807 $P < 0.05$ (1-tail)
No. of res. spaces ($/mm^2$)	.062 ±.046	.200 ±.109	1.6494 $P < 0.10$ (1-tail)

TABLE 7.- ASSIGNMENT OF LABELED OSTEONS TO BIRTH AT DIFFERENT TIME PERIODS

Period	Pre-1	1-2	2-3	3-4	Post-4
Duration (days)	--	9	13	9	7
Seam births (control)	17	6	1	4	0
Per animal	8.5	3	.5	2	0
Seam births (immobilized)[a]	88	18	3	4	0
Per animal	17.6	3.6	.6	2	0

[a]Note that the excess number of seams in the immobilized animals were present before the start of the experiment and therefore could not be the result of immobilization.

TABLE 8.- TOTAL NUMBER OF INDIVIDUAL LABELS OBSERVED IN CONTROL AND IMMOBILIZED ANIMALS, COMPARED WITH THE EXPECTED NUMBER

Label	1[a]	2[a]	3[a]	4
Control - observed	18	25	21[b]	10
- expected	21.5	21.5	21.5	10.25
Immobilized - observed	83	91	49[c]	42
- expected	87	87	87	35

[a]For labels 1, 2, and 3, this is calculated as the mean of the observed numbers for the first and second labels, with an appropriate correction for the fourth label for the numbers sacrificed at different times.
[b]X^2 = 1.1511 NS.
[c]X^2 = 16.9655 P < 0.001.

TABLE 9.- INDIVIDUAL MEAN VALUES FOR APPOSITIONAL RATE (μm/day) AT DIFFERENT STAGES OF THE EXPERIMENT (MEANS ±SE). NUMBERS OF OBSERVATIONS IN PARENTHESES.

	Label period				
	1-2	2-3	P <[a]	3-4	P <[a]
Control					
A220	0.855 ±0.041 (31)	0.812 ±0.041 (47)	NS	[b]	
A212	1.293 ±.087 (22)	1.034 ±.082 (36)	0.05	0.889 ±0.057 (37)	0.001
Immobilized					
72B	1.073 ±.086 (26)	.728 ±.053 (17)	.01	[b]	
A396	1.391 ±.087 (89)	.826 ±.040 (95)	.001	[b]	
A392	1.233 ±.068 (62)	[c]		[b]	
A350	1.112 ±.067 (48)	1.058 ±.056[d] (83)	NS	.727 ±.29[d] (87)	.001
48E	1.310 ±.108 (37)	.863 ±.066[d] (44)	.001	.838 ±.061[d] (44)	.001

[a]For comparison with (1-2).
[b]Sacrificed early.
[c]No third label.
[d]Include measurements between second and fourth labels.

TABLE 10.- GROUP MEAN VALUES FOR APPOSITIONAL RATE (μm/day) AT DIFFERENT STAGES OF THE EXPERIMENT.
[The labels between which the measurements were made are indicated by number pairs.]

	Control (C)					Immobilized (I)					P	
	n	Mean	SE	p[a]	%	n	Mean	SE	p[a]	%	C-I(Abs)[b]	C-I(%)[c]
1-2	53	1.037	0.053	--	100	200	1.230	0.053	--	100	--	--
2-3	83	.908	.044	<0.1	87.6	239	.906	.029	<0.001	73.6	<0.01	<0.01
3-4	37	.889	.057	<.1	85.7	131	.764	.028	<.001	62.1	<.001	<.001

[a]Significance of differences from (1-2).
[b]Significance of differences between immobilized and control for absolute decrements.
[c]Significance of difference between immobilized and control for percentage decrements.

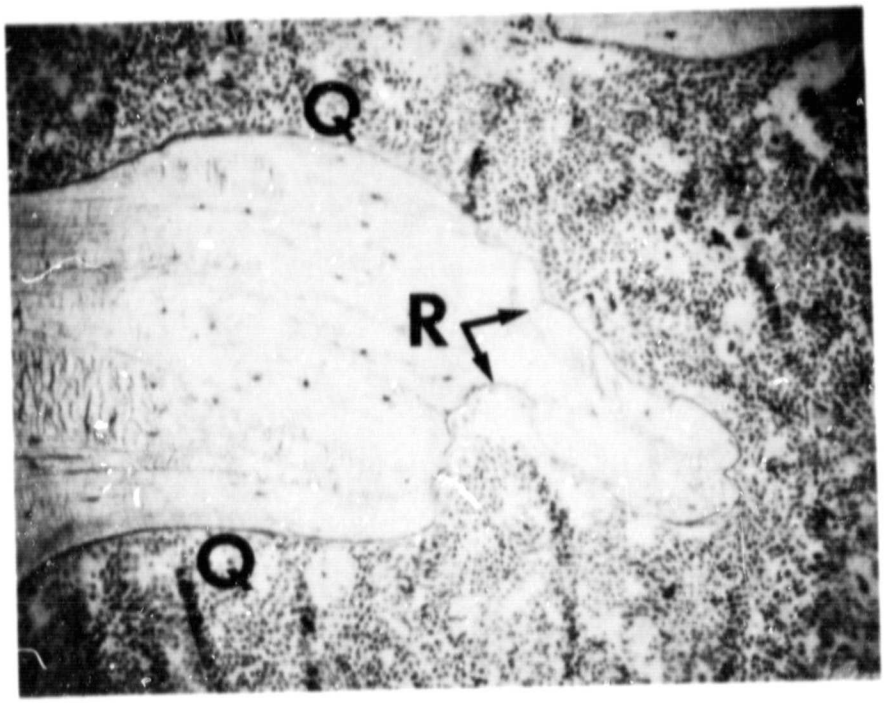

Figure 1.- Monkey 392, vertebra. Note area of deep resorption (R); (Villanueva Bone Stain, 80 X); Q = quiescent surface.

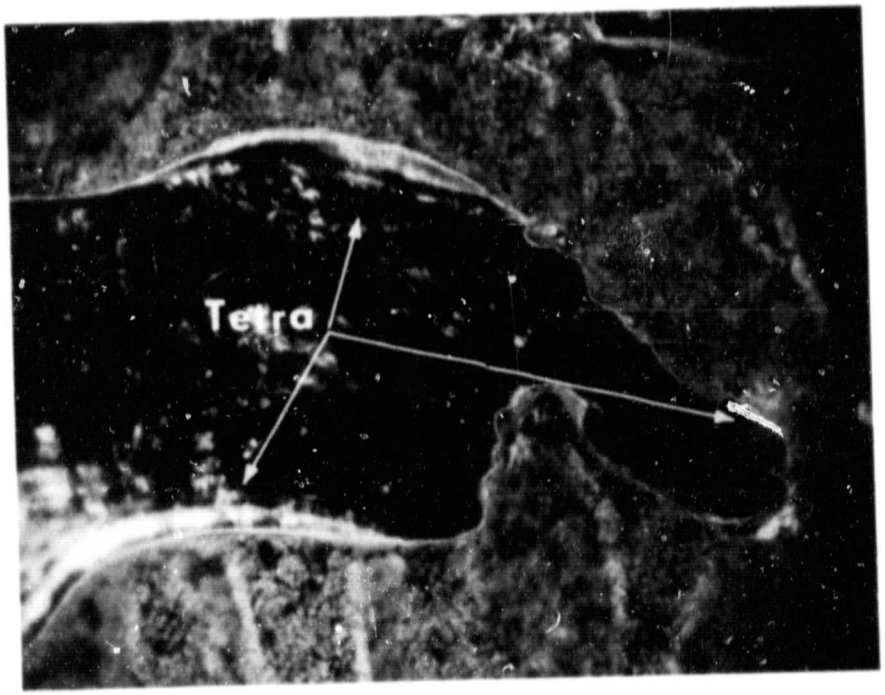

Figure 2.- Newly formed bone (as indicated by tetracycline (Tetra) bands) is being resorbed during immobilization (Villanueva Bone Stain, 80 X).

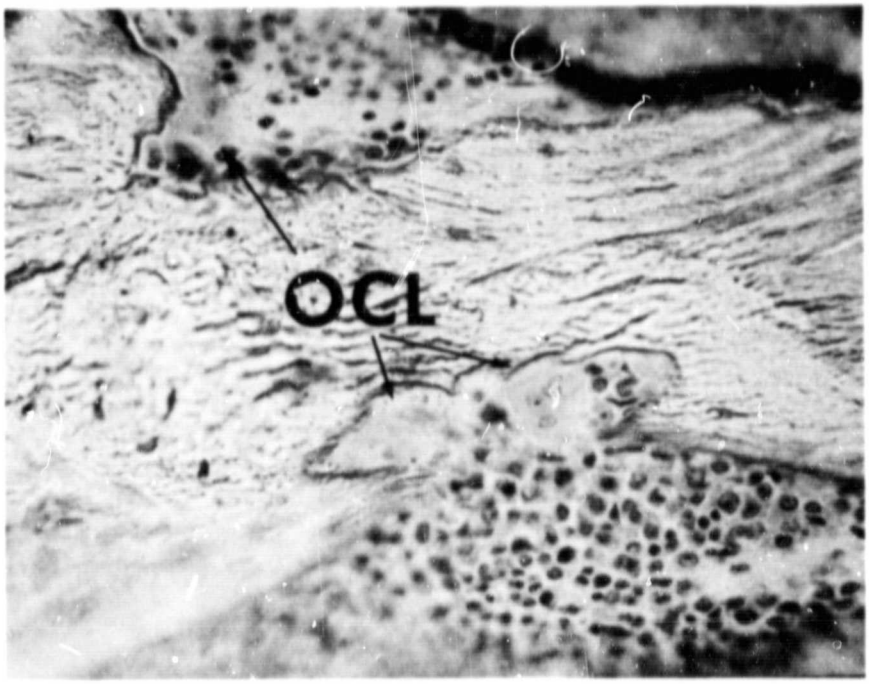

Figure 3.- Monkey 396, vertebra. Active resorption with osteoclasts (OCL) on both sides of this trabeculum (Villanueva Bone Stain, 200 X).

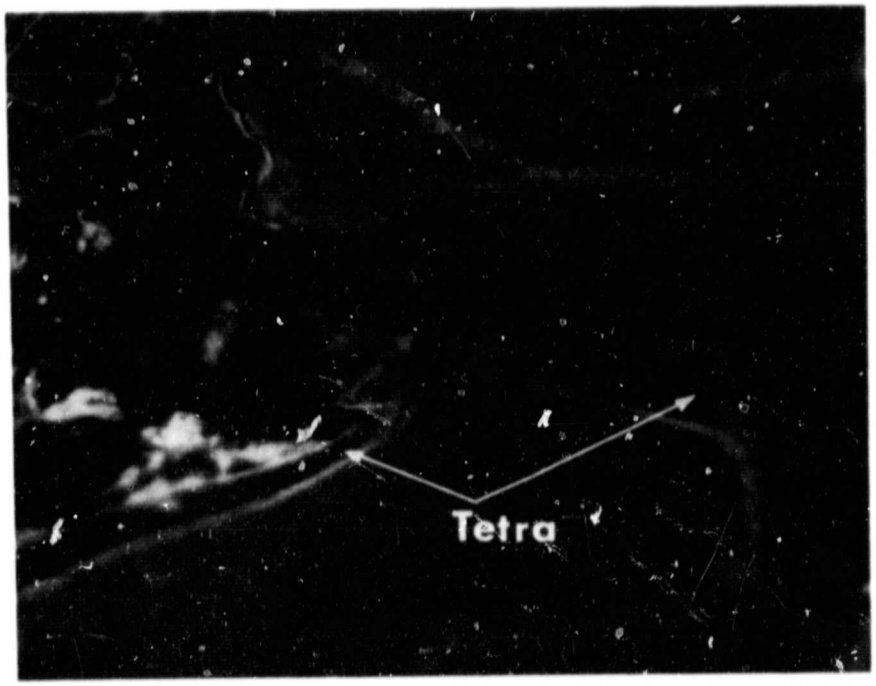

Figure 4.- Same as above, fluorescent light. Lower region of trabeculum is recently formed bone as indicated by pre-immobilization tetracycline (Tetra) label (Villanueva Bone Stain, 128 X).

CALCIUM METABOLISM AND CORRELATED ENDOCRINE MEASUREMENTS IN NONHUMAN PRIMATES DURING HYPOKINESIA

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INTRODUCTION

The underlying causes of bone loss during spaceflight and immobilization are unknown. In immobilized patients there appears to be an unexplained increase in bone resorption coupled with a decrease in bone formation. Spaceflight results from rat studies show a decrease in bone formation, but data are not yet available on bone resorption changes.

The dynamic bone changes which occur in rats during spaceflight occur early, probably within the first 3 to 4 days. Human data show that urinary calcium increases within 3 to 4 days after the onset of immobilization or spaceflight. A primary objective of this ground-based pilot immobilization study in monkeys was to define the magnitude and kinetics of the calcium metabolic changes which would be expected to occur during a 2-week spaceflight. It was hypothesized that even though no major gross changes (such as in bone mass) would be observed, the experimental design could be optimized to see easily any changes which might occur in the rates of bone formation or resorption and the associated metabolic and endocrine changes.

METHODS

These studies were done using seven male rhesus monkeys (Macaca mulatta, 6.4-10.4 kg). Serum samples were obtained at T-8, T+4, T+11, R+0(T+14), R+4, and R+14 days with T = 0 the beginning of immobilization and R+0 the end of immobilization. Ten-ml blood samples were drawn from experimental and control animals and allowed to clot at ambient temperature for 3 ±1 hr. Samples were then centrifuged at 4°C for 30 min, the serum was drawn off, placed in glass vials, and frozen at -20°C. Serum samples were analyzed for calcium, phosphorus, total protein, parathyroid hormone, 25(OH)D, and $1,25(OH)_2D$.

Serum calcium was measured using an automatic EDTA titration micro method (Calcette). The 25(OH)D measurements were done with a competitive protein binding assay with preliminary separation to remove interfering species. The PTH assay used for these studies has been developed recently and is not yet fully optimized for monkey PTH. However, it has been tested with both rhesus (M. mulatta) and pigtail (M. nemestrina) sera, and PTH from both species shows good cross-reactivity when compared to a human standard

of highly purified 1-84 hPTH. Sensitivity appears to be slightly better for pigtail than for rhesus, but work is continuing using hypoparathyroid monkey serum as diluent and PTH extracted from monkey parathyroid glands to optimize this assay.

PRELIMINARY RESULTS

Total calcium was measured in all samples. The immobilized animals showed a progressive decrease in total calcium from 9.6 mg/dl to 9.0 mg/dl throughout the immobilization period. There was a large increase to 11.3 mg/dl upon the remobilization of two of the five casted animals (the other three were killed at R+0), and a decrease to normal of 9.3 mg/dl by R+14. Two control animals studied during this same period showed a slight increase in serum calcium early. Changes in serum calcium from baseline are shown in table 1. The serum calcium changes do not appear to be due to a change in fluid concentration, since a diuresis rather than expansion of ECF normally occurs during immobilization. Total serum proteins are being determined and the serum calcium changes will be interpreted in terms of any protein changes as well.

Serum phosphorus was measured in all usable samples. Some samples were hemolyzed, and thus the phosphorus content could not be determined. In all but one animal, changes were noted with immobilization, with an increase of 0.4 to 1.3 mg% depending on length of casting.

The 25(OH)D levels have been measured in four of the seven animals, and PTH in three of the seven. These data are presented graphically in figure 1. The 25(OH)D values appear to reflect the changes in serum calcium. In the one animal in which Ca, 25(OH)D, and PTH changes were smooth (monkey A350), the Ca decreased along with 25(OH)D, and PTH increased slightly.

DISCUSSION

Previous work with restrained monkeys has shown that parameters of calcium metabolism as measured by calcium tracer kinetic methods show definite changes 3 weeks after the beginning of immobilization (table 2). The magnitude of these changes is such that after 2 weeks of spaceflight, the measurements of calcium turnover, absorption, and bone resorption should show significant changes from baseline. The preliminary data from this recent immobilization study show definite changes in serum calcium and suggest significant changes in serum 25(OH)D and PTH levels.

For all these experiments, each animal has been used as his own control. Even the use of weight/age matched pair-fed control animals cannot eliminate the individual variability in primate populations. This is important not only in the changes induced by spaceflight but also in the study of recovery after flight; endocrine and metabolic studies are much more meaningful if

the animals are not killed immediately after flight but are followed for 2 to 4 weeks.

The observation of metabolic changes with immobilization for even this very limited number of animals demonstrates the sensitivity of the techniques which are being used. The variability in the control animals demonstrates the necessity for several blood samples both pre- and post flight to establish the changes which occur. The techniques which have been used in this study are being modified and optimized as microanalytical methods so that smaller amounts of serum will be needed.

SUMMARY

Calcium metabolic and endocrine measurements show that changes do occur in a short time after immobilization, and that our sensitive techniques can detect these changes. The findings of an apparent depression in serum calcium early in immobilization and the large transient increase seen after remobilization were unexpected. These observed changes demonstrate the need for a correlated metabolic/endocrine/histomorphometric approach to the study of calcium metabolism in immobilization and spaceflight.

TABLE 1.- MEAN CHANGE IN SERUM CALCIUM FROM BASELINE VALUES

	T+4	T+11	R+0	R+4	R+14
Casted N = 5 →	0.01	-0.37	N = 2 → -0.58	+2.17	+0.11
Controls N = 2 →	+1.12	+1.28	N = 1 → -.16	+2.98	+.46

TABLE 2.- CHANGE IN CALCIUM METABOLIC PARAMETERS WITH IMMOBILIZATION (mg/day)

	Baseline	3-weeks restraint
Calcium turnover	302	438
Urinary excretion	116	123
Endogenous fecal	44	67
Calcium balance	+15	-9
Intestinal absorption	175	181
Resorption	127	257
Formation (kinetic method)	142	248

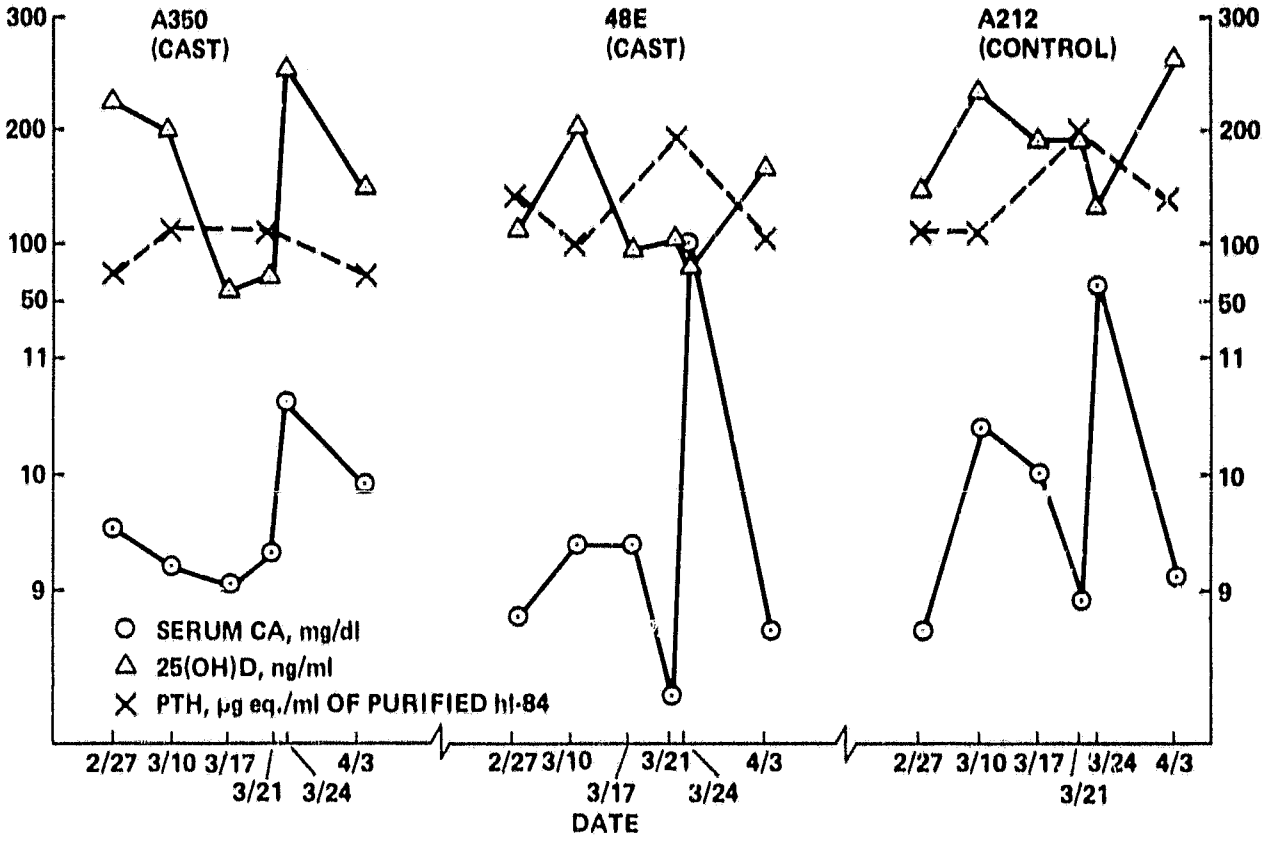

Figure 1.- Serum calcium and calciotropic hormones.

THE EFFECT OF POSTCRANIAL IMMOBILIZATION ON THE GROWTH OF THE RHESUS MONKEY JAW

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INTRODUCTION

In 1982, Soviet scientists plan to orbit a biosatellite with two to three rhesus monkeys on board. This flight offers the prospect that we can determine the biological basis for the losses of skeletal mass that have been observed in the astronauts who participated in the USA Skylab missions (28 days, 59 days, and nearly 3 months duration). These individuals who were studied before (21-31 days), during, and after flight (17 days) experienced some of the metabolic changes that caused negative shifts in calcium balance (-184 milligrams/d) and increased bone resorption. Some of these changes were doubtless attributable to the influence of null gravity per se and/or restricted motor activity, and the overall patterns of bone loss were not unlike those accompanying bedrest. In the COSMOS flight experiments involving young adult rats, null gravity in and of itself appears to be associated with a reduction in appositional bone growth (tibias), a slightly decreased level of bone resorption (whole body), and impairment of maturation of forming matrix and mineral moieties (jaws, refs. 1-8). Thus, spaceflight appears to have deleterious effects on the weightbearing bones (appendicular skeleton) and nonweightbearing mandibular elements. One must keep in mind that we cannot yet completely disassociate the effects of null gravity from the effect of reduced motor activity in generating the osteopenia common to mammals following "nonuse" of skeletal parts, for example, bed rest, casting of limbs, etc. In one sense, the situation during spaceflight has its parallel in hibernating animals which suffer episodic low calcium intake, evoking a resorptive parathyroid response; and many species, despite compensatory calcitonin secretion, become hypercalcemic, hyperphosphatemic, and demonstrate increased urinary and fecal calcium levels (ref. 9). One recalls that the rats orbited in the COSMOS biosatellites studies do not show increased surfaces of bone (tibias) covered by cells with rich acid phosphatase concentrations ("osteoclast") (ref. 2), and the thyroids have many more C-cells (calcitonin-producers) than normal (ref. 10). It has yet to be demonstrated, however, that all of the cellular events observed in the long bones during spaceflight are present in every skeletal tissue.

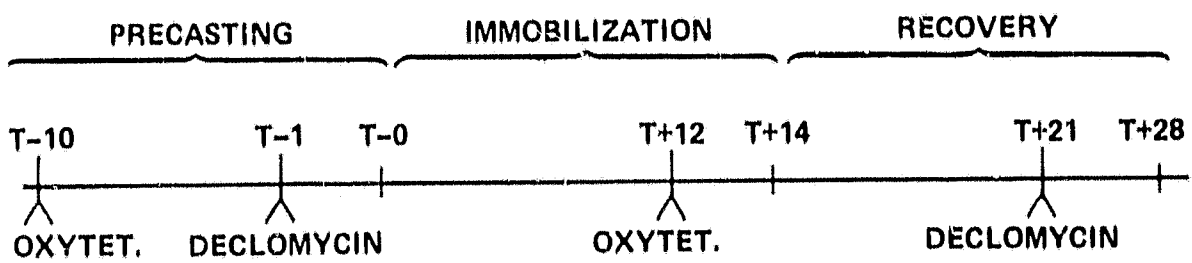
Thus, there may be reason to believe that the effects of spaceflight on the skeletons of animals and man are separate from the effects of reduced physical activity. It is essential that the evaluations be extended to primates if the work is to be truly meaningful for man. The present study deals with the effects of immobilization on rhesus monkeys as a prelude to the use of these animals as passengers in a biosatellite experiment. We have examined

the rates of periosteal, endosteal, and osteonal growth (and closure) in the jaws of animals that were subjected to full-body (posteranial) casting. In the experiment, the mandibles remained fully functional.

MATERIALS AND METHODS

The studies involved seven rhesus monkeys (6-10 kg). Two of the animals were maintained as controls in metabolism cages. Five were placed in full body casts and positioned with their limbs and trunk upright. The immobilized monkeys were not moved during the experimental period of 14 days. All the animals were exposed to a 12 hr/12 hr light-dark cycle in a windowless air-conditioned room, at a temperature of 76 ±2°F. The weights of the control and experimental monkeys were recorded for 10 days (T-10 to T-1 days) prior to immobilization (T-0) and after decasting (T+14 days). Several animals were killed after decasting (A396, 72B, A220); the others (A212, A350, 48E) were allowed to recover in metabolism cages. During the full period of the study, the animals were fed a standard diet of Purina Monkey Chow and fruit (3X/d); water was available ad-lib. Food and water consumption was monitored and recorded.

In order to mark sites of bone formation during the three periods of study, 20 mg/kg oxytetracycline (green fluorescence) and declomycin (yellow fluorescence) was administered i.v. before, during, and after immobilization. We alternated use of these antibiotics in order to assure identification of the packets of bone formed during each of these periods. The schedule was as follows:



All the monkeys were sacrificed by an i.v. overdose of euthol (1 ml/5 lb body weight).

At autopsy, the skulls and jaws were resected and fixed in 100% ethyl alcohol. The bones were subsequently stripped of soft tissues. Blocks of bone were cut from the condylar, lingular, molar, and premolar regions with a Striker oscillating saw (fig. 1). These regions were then embedded undecalcified in methylmethacrylate, and sectioned transversely on a high-speed rotary saw at 50 µm. The sections were then examined by UV microscopy to identify the tetracycline labeling patterns and to establish the most optimum sampling sites. Each section was then microradiographed on high resolution

Kodak 649-0 spectroscopic plates (10 kV, 20 mA, 10 min) to reveal the microscopic distribution of mineral densities in the bone. These preparations were compared to the unstained sections (used for UV microscopy) to control the sites at which measures of the appositional bone growth and osteon closure rates were taken.

Osteon Frequencies

The initial surveys of the sections indicated that osteon populations were richest at the lingula. Fewer osteons per section were present in the bony walls of the jaw at the level of the premolars and molars. Osteons were insignificant components of the jaw at the level of the condyle and M_3.

Tetracycline Labeling Patterns

UV microscopy demonstrated the osteons which incorporated tetracycline after each injection period. In order to estimate the number of osteons growing during the pre-immobilization, immobilization, and recovery periods, we estimated the percentage of osteons which bore each of the four labels. However, because it was so difficult to properly identify singly labeled osteons when the label lay at the mineralization front, these data were not recorded. Basically, we could not distinguish between the autofluorescence due to bone mineral itself and the fluorescence of this narrow zone of recently formed bone mineral. We encountered fewer identity problems when osteons were multiply labeled, and here we calculated the specific rates of appositional bone growth and the relative rates of osteon closure.

The rates of bone growth during the precasting, the casting, and the recovery periods were then estimated using an ocular micrometer at a magnification of 125 X. We developed specific information about bone formation at the lingular and premolar (PM_1) regions. Again, too few (useful) numbers of osteons were present in the cortical bone in the condylar and premolar regions. All measurements were taken from the center of one label to the center of the next. Only osteons which failed to demonstrate hypercalcified growth arrest lines were used (fig. 2). These values were then divided by the time interval between injections so that the growth rates could be expressed in terms of μm/d. These data, however, could not provide truly meaningful estimates of appositional growth because they assume a unified growth rate from the beginning to the end of osteon formation — and we know from other sources (refs. 9, 11, and 12) that osteon growth slows as these systems become complete. Thus, an error in interpretation might arise if, for instance, osteons in the controls were labeled predominately in the stages of early formation, and if the osteons in the immobilized group were labeled predominately toward the ends of their growth period. Consequently, we evaluated the radial rate of osteon closure during the pre-immobilization period in all seven monkeys, whether or not they were entered into the immobilization group, and during the immobilization period in the five casted monkeys. The method used was that described by Manson (refs. 11, 12) in a number of studies on growing adult dogs and cats. In those osteons labeled

in two successive labeling periods, for example, T-10 to T-1d, or T-1 to T+12d, or T+12 to T+12d, the diameter of the outer fluorescent ring (measured microscopically at 125 X) was plotted against the diameter of the inner fluorescent ring, and the data were analyzed by least squares analysis to determine the slope of the regression line. It became apparent during the course of these analyses that a significant number of osteons in the immobilized monkeys failed to grow during the immobilization period; their osteons had not incorporated the oxytetracycline label administered just before decasting (T+12 days). This observation severely limited the size of the osteon sample in the immobilized group, and rendered tentative the estimate of the osteon closure rates. Accordingly, any conclusions about the differences in the calculated rates of osteon closure in these two groups of animals must be tempered with caution.

The paucity of labeled osteons in the immobilized monkeys also hampered efforts to derive information about comparative sizes and diameters of "remodeling units." For instance, a decrease in bone formation rate might be signaled by a decrease in the mean wall thickness or osteon diameter, and an increase in the average Haversian canal diameter.

Osteon Size

Because the soft tissues in the Haversian canals made it difficult to precisely locate the (endosteal) borders of the most recently formed lamellae of bone, we used the microradiographs to estimate the diameters of the osteons, the diameters of the Haversian canals, and the mean osteon wall thickness. Concentric osteons were measured on two axes, 90° to one another. Eccentric osteons, which were most prevalent in the premolar regions, were measured on their long and short axes. The data were averaged for each osteon. We note that while the use of microradiographs was necessary, the data tended to overestimate the true diameter of Haversian canals. This is because these preparations do not record the most recently formed inner layer of unmineralized bone (osteoid). It should also be noted that this exigency could affect our conclusions. If, for instance, immobilization were to (1) stimulate bone formation and initially interfere with calcification, and (2) secondarily decrease bone formation (and, thereby, permit the osteoid previously formed, e.g., during the pre-immobilization, to mineralize), it might appear that the mean wall thickness was normal. Conversely, if immobilization were to decrease the bone formation rate and interfere with calcification of previously formed osteoid, the estimation of mean wall thickness would be significantly underestimated and the estimated diameter of the Haversian canals would be greater than expected. Despite these caveats, it will be seen that these aspects of bone histomorphometry were not significantly affected by immobilization.

Statistics

The grouped data for the control and immobilized rhesus monkey bones were compared by the Students t-test when the variances were equal. When the

variances were unequal, the significance of the data was estimated by a non-parametric F-test.

RESULTS

The data for most of the monkey jaws at the level of the lingula and premolar have been compiled in tables 1 and 2. In some instances, such as control monkey A220 or casted monkey 72B, the bones contained so few regions of tetracycline uptake that we could not, reliably, compute their rates of bone formation or labeling frequency.

The numbers of osteons in the lingular and premolar regions in the jaws from individual monkeys which were growing when each of the fluorochrome labels were administered are recorded in table 2. The total number of osteons is not large (average of four sections each), but it is clear from table 1 that 39% of the osteons in the control rhesus (A212) bore four labels, one corresponding to each injection, and that the remaining 61% of osteons were labeled two or three times. On the other hand, most of the osteons in three of the five immobilized animals only bore evidence of one or two injections (89%), and these were most commonly the labels administered during the pre-immobilization period (T-10 and T-1). Thus, the data suggest that the third label administered just prior to decasting in the immobilized animals was absent in many of the casted animals.

These observations suggest that the test animals experienced fewer osteons "starts" during the period of immobilization. Nevertheless, the rates of bone growth were not unusual in those osteons which continued to grow during the casting period. Table 3 indicates that, on the average, osteon growth ranged between 1.3 and 1.5 μm/d during these periods, while growth on the periosteal surface ranged between 1.2 and 1.3 μm/d. The data for the endosteum seem to suggest that bone growth increases during casting, but the control data (A212) are so few as to make this interpretation hazardous. Very little growth was experienced during the postcasting recovery period (1 μm/d) in any of the immobilized animals studied, and the decrease from the casting period was from 30 to 70%. The microradiographs record what appears to be an increase in the development of growth arrest lines in labeled osteons from two immobilized animals, but there are too few control data to properly evaluate the observation (table 1).

Osteon Size

Table 4 indicates that, on average, there were no significant differences between mean diameters of the osteons in the jaws from the control (152.2 ±9.7 μm) and immobilized (175 ±16.8 μm) monkeys. The average diameter of the Haversian canals was also similar (control = 65.1 μm; immobilized = 75.3 ±14.1 μm). The distribution of the values for the individual rhesus monkeys was sufficiently great to obviate comparisons during the immobilization and recovery periods.

Osteon Closure Rates

When the diameters of the tetracycline markers from osteons which showed labels from two or more successive tetracycline injections were plotted (radius of the outer label versus radius of the inner label) and regression lines calculated, the slopes varied between 0.791 and 0.997 (table 5). Most of the data were drawn from osteons in the lingular cortex where the form of the osteons was predominately concentric. Figure 3 displays the grouped data for all seven animals during the pre-immobilization period (labels injected at T-10 and T-1 days) and for the controls during the immobilization and post-immobilization recovery (T+12 and T+21 days). This plot is contrasted with data from three rhesus monkeys calculated during the immobilization period. Despite the paucity of data for the immobilization period in the casted monkeys, the slopes of the regression lines were not statistically different. This again suggests that the pace of appositional growth in those osteons which continue to grow is entirely within normal limits.

DISCUSSION

The salient observations from these studies are:

1. Immobilization by whole body (postcranial) casting — even when the jaws are free to function and do function in mastication — does cause a decrease in the formation of mandibular Haversian bone.

2. Recovery may be incomplete as long as 14 days after decasting. These observations are based upon the preponderance of osteons in the lingular and premolar regions of the jaw which fail to label with tetracycline during the immobilization period and after decasting.

3. Bone formation on periosteal and endosteal surface of bone and in those osteons that do recover and grow appears to proceed at a normal pace. But this conclusion is really based upon an inadequate sample size (re: osteon dimensions, labeled osteons, etc.). We note, particularly, that the labeled osteons in the immobilized monkeys tended to be "larger than normal" and have wide Haversian canals — observations which might result from an initial increase in resorption and impaired internal reconstruction (appositional bone growth).

Thus, these results are quite preliminary, and it would not be appropriate to draw any firm conclusions about the condition of appositional bone growth in the jaws as a whole — or to extend these findings to elements of the skeleton which play a significant role in weightbearing. We can safely say that there do not seem to be important statistical effects of axial and appendicular immobilization on the lingular and premolar regions of the mandibular cortex. This result might have been anticipated since the bone remained functional throughout the study. Because these results might have been influenced by the small sample size, the possibility remains that, with a more extended study, definitive changes due to postcranial immobilization

will appear in the jaws. If, for instance, the lingular and premolar regions were regions of relatively slow bone turnover, it would be difficult to discern large changes due to any systemic effects of the immobilization (endocrine changes). These changes would become more obvious in regions of the jaw that were metabolically more active. Nevertheless, these tissues deserve careful attention because the rat data (COSMOS 1129) indicate that there are disassociative effects of spaceflight and motor activity. An inability to discern a change in bone architecture during immobilization does not preclude the fact that some change will become evident when rhesus monkeys are flown in biosatellite experiments.

SUMMARY

The effect of postcranial immobilization on bone growth in the rhesus monkey mandible was estimated by tetracycline labeling and microradiographic techniques. The animals had received fluorochrome markers at the beginning and end of a pre-immobilization control period (10d), at the end of 14-day casting period, and on the seventh day after decasting (recovery period). Postcranial immobilization reduced the labeling frequency in the osteons from the lingular and premolar (PM) regions of the mandible during the casting and recovery periods. In control bones (one of two animals), 70% of osteons bore each of the four tetracycline markers, while in three of five immobilized monkeys the bone was labeled largely by the injections administered during the control period (86% osteons). About 10% of the labeled osteons in two of five immobilized monkeys showed hypercalcified growth arrest lines. The treatment did not, however, seriously retard the rate of appositional bone growth in those osteons which continue to grow during the periods studied. Osteon dimensions were unaffected by the protocol. From these quite preliminary studies, we conclude that functional elements such as the mandible do not escape the deleterious effects of postcranial immobilization, and that the major effect of treatment is to retard the osteon birth rate.

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TABLE 1.- DISTRIBUTION OF TETRACYCLINE MARKERS IN OSTEONS IN THE RHESUS MONKEY JAW. EFFECT OF POSTCRANIAL IMMOBILIZATION

Group	Number of osteons	Number of osteons labeled 1X to 4X				Osteons with growth arrest lines	
		1X	2X	3X	4X	Number	%
Control							
A212	13	--	4	4	5	0	0
Immobilized							
A396	17	--	13	4	0	2	11.7
72B	3 (PM_1)	--	2	1	0	0	0
A392	11	--	11	0	0	0	0
Immobilized and recovery periods							
A350	20	--	19	0	1	3	15.1
48E	2	--	2	0	0	--	--

Percent labeled osteons

	1X	2X	3X	4X
Control	--	30.7	30.7	38.5
Immobilized	--	88.7	15.2	1.9

TABLE 2.- INCIDENCE OF TETRACYCLINE MARKERS IN OSTEONS IN THE RHESUS MONKEY JAW. EFFECT OF POSTCRANIAL IMMOBILIZATION

Group	Number of osteons	Injection times (days)			
		T-10	T-1	T+12	T+2
Control					
A212	13	2 3 5 2 1			
Immobilized					
A396	17	13 4			
72B	3	1 2			
A392	11	11			
Immobilized and recovery periods					
A350	20	1 19			
48E	2	2			

TABLE 3.- APPOSITIONAL BONE GROWTH IN THE RHESUS MONKEY MANDIBLE (μm/d)

	Rhesus number	Group		Precast period	Casting period	Recovery period
Lingula osteons	A212	Control		1.06	1.30	0.62
	A220	Control		—	—	—
	72B	Casted		—	—	—
	A396	Casted		1.79	1.27	—
	A350	Casted		1.43	1.43	0.42
	A392	Casted		1.20	1.08	—
	48E	Casted		—	—	—
			Mean ±SE	1.47 ±0.20	1.26 ±0.12	0.42
Lingula periosteum	A212	Control		1.58	0.79	—
	A220	Control		—	—	—
	72B	Casted		—	—	—
	A396	Casted		1.7	1.24	—
	A350	Casted		1.1	1.12	—
	A392	Casted		0.84	1.6	—
	48E	Casted		—	—	—
			Mean ±SE	1.21 ±0.29	1.32 ±0.16	—
Lingula endosteum	A212	Control		0.67	0.97	0.51
	A220	Control		—	—	—
	72B	Casted		—	—	—
	A396	Casted		1.28	1.13	1.56
	A350	Casted		1.36	1.12	0.08
	A392	Casted		1.21	1.16	—
	48E	Casted		—	—	—
			Mean ±SE	1.28 ±0.05	1.14 ±0.01	0.82

TABLE 4.- OSTEON SIZE IN RHESUS MONKEY JAWS.
EFFECTS OF POSTCRANIAL IMMOBILIZATION

Rhesus number	Controls (μm)		Immobilized (μm)	
	Osteon diameter	Canal diameter	Osteon diameter	Hav. Canal diameter
A212 L	152.2 ±9.7	65.1 ±12.3		
A220 L, PM_1	--	--		
Immobilized				
A396 L			205.6 ±22.0	103.4 ±29.7
PM_1			190.8 ±22.7	118.8 ±9.5
72B L			104.2 ±6.7	33.8 ±6.3
A392 L			154.6 ±14.9	74.3 ±12.2
Immobilized and recovery periods				
A350 L			198.0 ±17.6	89.5 ±8.8
PM_1			199.3 ±34.5	106.5 ±10.0
48E L, PM_1			--	--
Mean ±SE	152.2 ±9.7	65.1 ±12.3	175.5 ±16.8	75.3 ±14.1

L = lingular region
PM_1 = region of the first premolar

TABLE 5.- THE RATES OF OSTEON CLOSURE IN THE RHESUS MONKEY JAW. EFFECT OF POSTCRANIAL IMMOBILIZATION

Group	Rhesus number	Osteons			
		Lingular cortex		Premolar cortex	
		Slope	Intercept	Slope	Intercept
Control	A212	0.884	-1.57	--	--
	A220	--	--	--	--
Immobilized	A396	.997	-9.88	1.008	-13.92
	72B	--	--	--	--
	A392	.863	-1.11	--	--
Immobilized and recovery periods	A350	.791	-1.05	.930	-5.50
	48E	--	--	--	--

SUMMARY

Group	Number of osteons	Slope	Intercept
Control rhesus and pre-immobilization periods	55	0.880	-3.82
Immobilization period	7	.896	-6.19

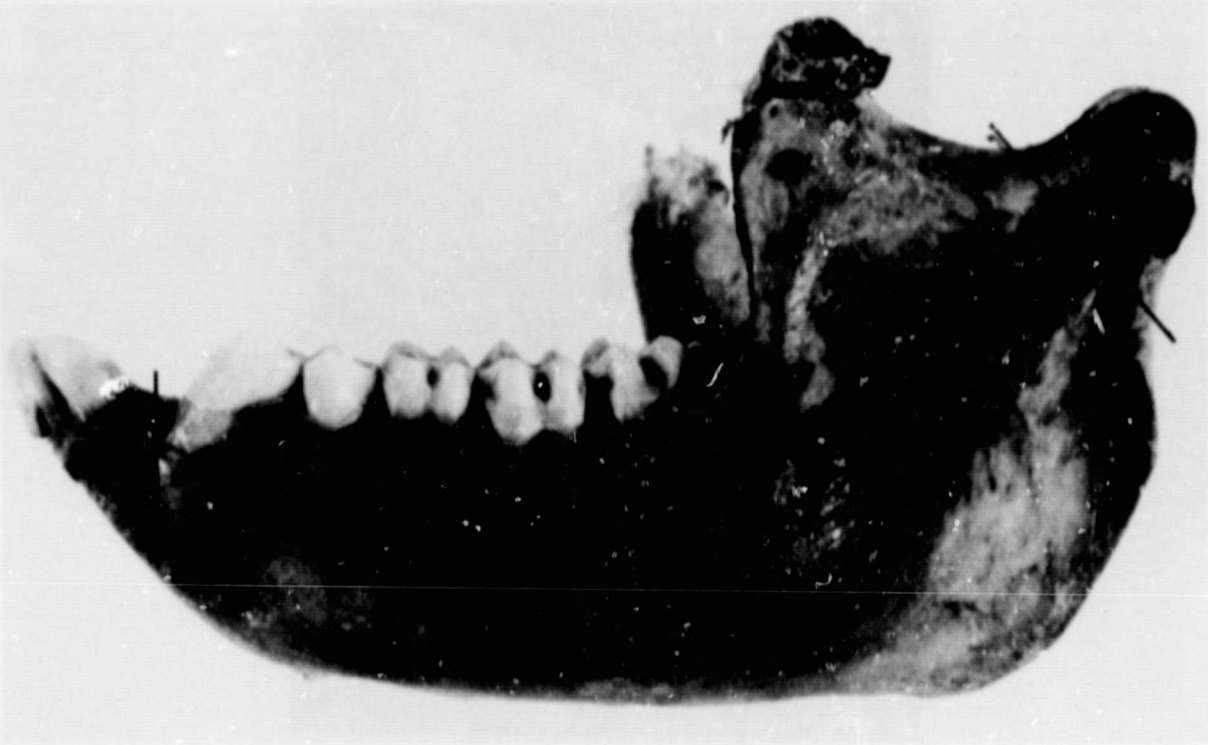

Figure 1.- Lateral view of the 6-kg rhesus monkey jaw showing areas from which blocks of bone were cut for analysis of appositional growth patterns. A = Condyle, B = Lingula, C = Premolar (PM_1), D = Molar.

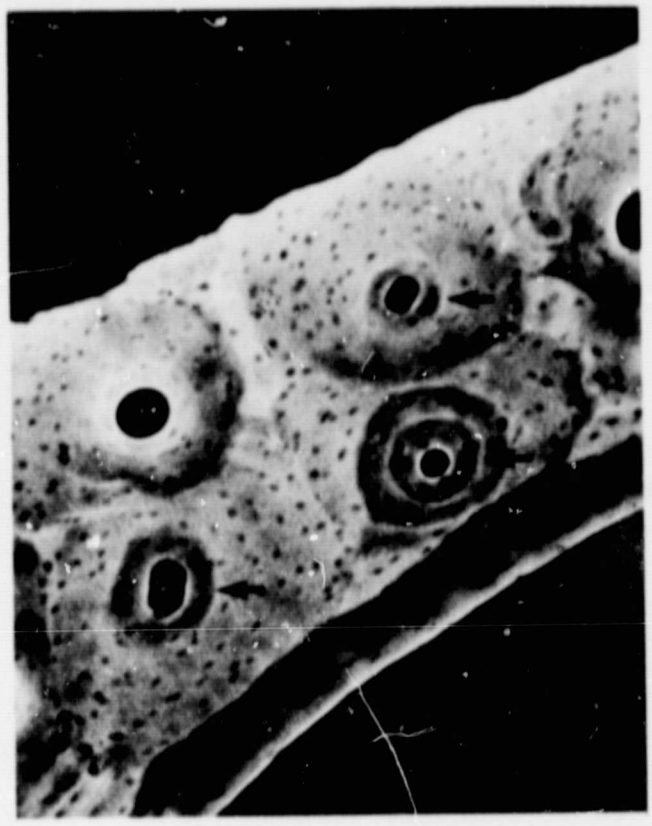

Figure 2.- Microradiograph of an undecalcified section from an adult human rib showing osteons bearing hypercalcified growth arrest lines (arrows).

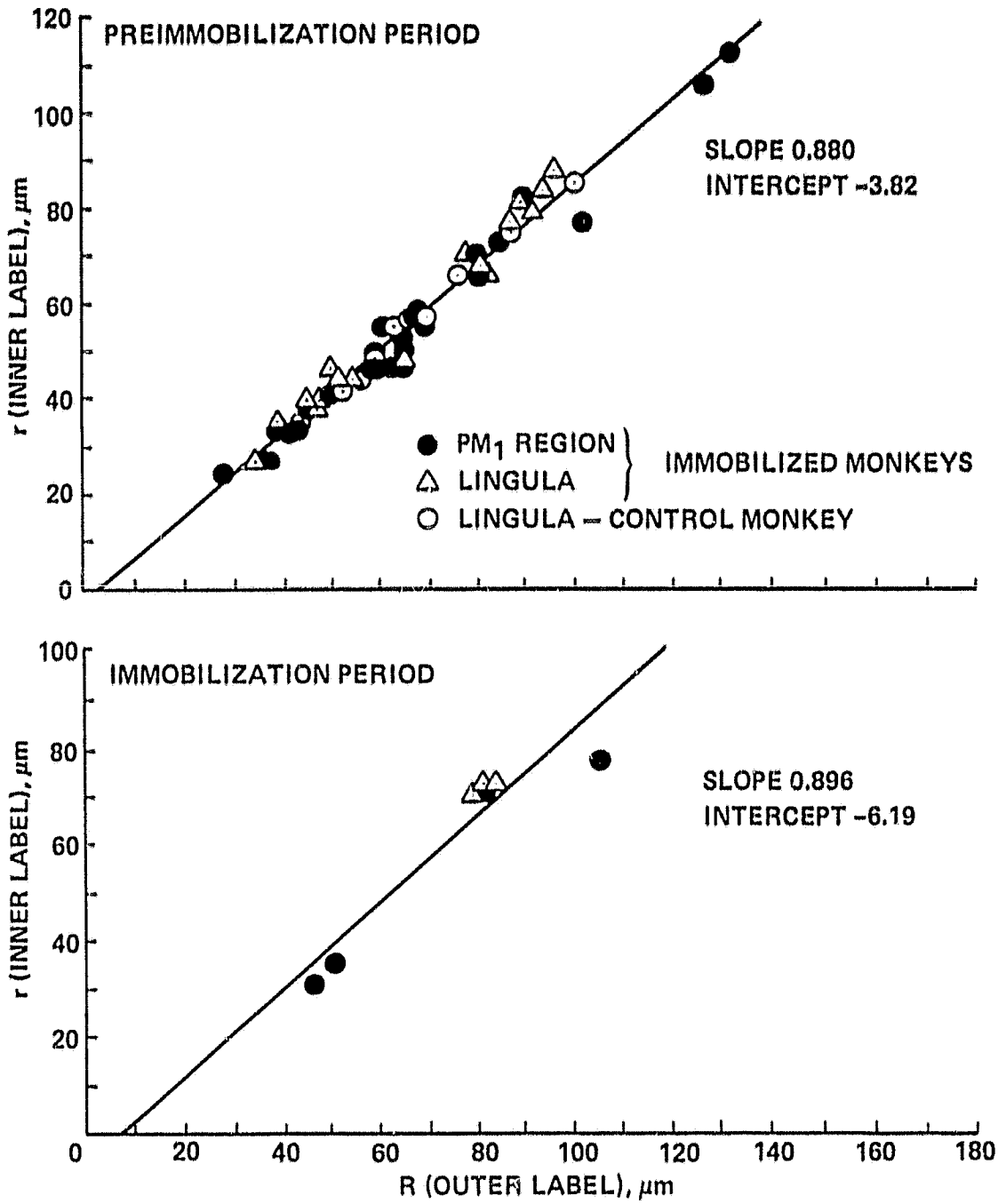

Figure 3.- Radius of inner label plotted against radius of the outer label from osteons in mandible (Lingula and Premolar regions) of rhesus monkeys. Slopes of regression lines do not differ significantly, suggesting that the radial rate of osteon closure is similar during control and immobilization periods.

THE EFFECT OF POSTCRANIAL IMMOBILIZATION ON THE MATURATION OF MATRIX AND MINERAL MOIETIES IN THE RHESUS MONKEY JAW

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INTRODUCTION

Because astronauts tend to lose bone mass during the weightless conditions of spaceflight and during bed rest/immobilization (at 1-G), it is commonly considered that the latter can provide a valid model system by which to study the effects of null gravity. Even laboratory rats show deficits of bone mass and strength (appendicular and axial skeletons) when they are immobilized or flown in space (refs. 1-3).

In the recent COSMOS 1129 Biosatellite flight involving rats (ref. 2), we had an opportunity to extend our investigations to the mandibles — a nonweightbearing skeletal element. Unlike the long bones, appositional bone growth in the jaw was not impaired, and the concentrations of calcium, phosphorus, magnesium, and hydroxyproline were normal. However, we did determine that spaceflight caused a decrease in the maturation of the mineral and matrix moieties. On the basis of specific density gradient fractionation, a much greater than normal percentage of the mineral and hydroxyproline resided in the lower density, newly formed fractions (1.3-1.9, 2.0-2.1 sp. gr.) than in the higher density, most mature fractions (2.2-2.9 sp. gr.). These analyses then suggested that density fractionation studies might be used to dissociate the effects of null gravity from the effects of reduced motor activity in generating the osteopenia seen after spaceflight or after bedrest/immobilization.

We recently had an opportunity to test this proposition in a 14-day hypokinesia study involving rhesus monkeys (ref. 4). On the basis of tetracycline labeling studies, the mandibles from the immobilized (whole body casts) rhesus monkeys exhibited a decrease in the numbers of growing osteons, but those systems which were growing during the study did so at a normal rate. Thus, we concluded that posteranial immobilization produced deleterious effects in both the nonweightbearing bones and in the weightbearing long bones and vertebrae. In this report, we present data detailing the distribution of calcium (Ca), phosphorus (P), and hydroxyproline (OH-Pr) from the mandibles in the region of the first premolar tooth. This region was chosen for two reasons. First, it has a relatively larger proportion of lamellar

bone than osteons. Second, much more of the bone in this region of the jaw had been labeled with tetracycline at some stage of the 40+ day study than in an osteon-rich bone region such as the lingula. The details of this experiment are provided in full in the original report (ref. 4), and the methods used were detailed in the COSMOS 1129 final report (ref. 3).

In this report, we also present similar data from three rhesus monkeys from a Soviet colony which were subjected to an 11-day period of immobilization in a head-down posture (6°). A head-down attitude achieves many of the fluid-electrolyte responses which have been observed during gravity unloading.

RESULTS

Total Mandibular Mineral-Collagen Content (tables 1-3)

Overall, the total concentrations of mineral and matrix hydroxyproline (OH-Pr) seemed not to be affected by immobilization in an upright of head-down posture. Total Ca, P, and OH-Pr varied from 148 to 197 mg/g, 82.3 to 87.8 mg/g, and 28.4 to 32.65 mg/g, respectively.

Density Gradient Fractionation (tables 1-3)

The percent of the total Ca, P, and OH-Pr which was contained in the most mature-highest density fractions (2.2-2.9 sp. gr.) of the mandibular cortex from the immobilized (U.S.A.) animals was generally within the normal range (56.6-29.3%). Only one immobilized U.S.A. animal (A396) and two of the three Soviet animals (SRh-2&3) had substantially lower values (18.91-21.96%). On the other hand, all the U.S.A. and Soviet animals which had been immobilized exhibited lower than normal concentrations of matrix and mineral moieties in the most recently formed-lowest density fractions. These animals also had higher than normal concentrations of calcium, phosphorus, and OH-Pr in the intermediate 2.0 to 2.1 sp. gr. fractions. The data for each animal are presented in their raw form in tables 1 to 3. It was not useful to try to express these results statistically since the groups of animals were very small.

DISCUSSION

The main objectives of this study were to determine if (1) there were changes in the mineral and matrix moieties in the mandibles of rhesus monkeys during postcranial immobilization, (2) whether any of these changes would be reversed during remobilization, and (3) how these changes compared to those observed in the mandibles of rats flown in the COSMOS 1129 Biosatellite (18.5d at 0-G).

First, the data suggest that immobilization in an upright posture or at a 6° head-down tilt produced fairly equivalent responses. In the immobilized

animals, there was a normal amount of mineral and OH-Pr in the actively growing lamellar bone of the jaw (PM_1 region). Moreover, there did not seem to be an important difference in the percentage of these moieties in the most mature density fraction of the bone. The most instructive data lay in the lowest two density fractions where there was, overall, a decrease in those moieties in the most recently formed tissues (1.3-1.9 sp. gr.), and an increase in the maturing tissue (2.0-2.1 sp. gr.). This was true for 6/6 animals in terms of Ca, and 5/6 animals in terms of P and OH-Pr. The rhesus A350, which was sacrificed two weeks after decasting, also had a fairly normal percentage of P and OH-Pr (and perhaps Ca), suggesting that its skeletal cells had had time to recover somewhat from the effect of immobilization.

The decrease in the lowest density fraction would be consistent with the observed reduction in mandibular osteon birth rate. Because those tetracycline data also indicated that the rate of appositional growth remained normal in osteons which grew throughout the immobilization period, it was to be expected that the concentrations of mineral and matrix would be normal in the most mature-highest density fraction. It was in the intermediate-maturing fraction (2.0-2.1 sp. gr.) that there was evidence of a delay in the rate of maturation of the bone matrix and mineral. These observations may be important when one considers the extent to which the immobilization model can be applied to problems of skeletal disease in astronaut populations. If immobilization were a "perfect" model for bone at null gravity, we would have anticipated — on the basis of data from the COSMOS 1129 rat studies — a decrease in percentages of Ca, P, and OH-Pr in the most mature density fractions (2.2-2.9 sp. gr.), and a marked increase of these elements in both the 1.3 to 1.9 and 2.0 to 2.1 sp. gr. fractions.

It would be inappropriate at this time to construct a broadly generalized statement about how the primate immobilization model may or may not be applicable to the study of osteoporosis (astro-osteoporosis) in astronauts. There are elements in common: (1) a suspected decrease in bone formation, (2) an uncoupling of bone formation versus resorption, such that the rate of resorption is relatively greater than the rate of formation, and (3) decreased bone strength. Our data have also indicated some diminished bone growth in non-weightbearing bones (the mandible). At this juncture, we can ask if there are elements of bone physiology which are not species related. It is tempting to "cross species lines" (rats to rhesus to man) and suggest that the responses to immobilization (in rhesus monkeys) will not mimic in every respect the changes seen in the skeletons of rats exposed to the 0-G conditions of spaceflight. The only argument we can raise to defend this position is that there is dissimilarity in the maturation of the matrix-mineral moieties in the mandibles of immobilized rhesus monkeys versus the COSMOS 1129 rats. This argument can only be resolved when samples of bone (weightbearing versus nonweightbearing bones) are procured from primates subjected to spaceflight.

SUMMARY

The effect of 14-day postcranial immobilization on the maturation of bone mineral (calcium = Ca and phosphorus = P) and matrix (hydroxyproline = OH-Pr) moieties in the rhesus monkey mandible was estimated by density gradient fractionation. There was no apparent change in total mandibular Ca, P, and OH-Pr in the bone from the immobilized animals, and the concentrations of these moieties in the most mature-highest density fraction (2.2-2.9 sp. gr.) was also normal. However, the concentrations of these moieties were lower than normal in the most recently formed-lowest density fraction (1.3-1.9 sp. gr.) and higher than normal in the maturing-intermediate density fraction (2.0-2.1 sp. gr.). The data suggest that 14-day immobilization in a primate species does not effect the same overall changes in bone-matrix maturation that were observed in rats which had experienced conditions of 0-G in the COSMOS 1129 Biosatellite.

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TABLE 1.- BONE CALCIUM CONCENTRATION IN RHESUS MONKEY JAWS. EFFECTS OF POSTCRANIAL IMMOBILIZATION

Rhesus number	Body weight (kg)	Total calcium (mg/g bone)	Density gradient fractions (sp. gr.) (% of total calcium)		
			1.3-1.9	2.0-2.1	2.2-2.9
Control (USA)					
A220	8.2	194.27	30.13	13.24	56.65
A212	6.5	149.04	20.12	10.58	69.30
Immobilized 14d (USA)					
A396	7.7	170.68	13.20	56.84	29.96
72B	7.7	169.77	12.39	24.46	63.15
A392	--	175.66	17.43	21.26	61.30
Immobilized 6° head-down 11d (USSR)					
SRh1	9.1	160.33	15.67	17.40	66.93
SRh2	5.1	154.39	16.68	57.10	26.23
SRh3	5.1	194.43	12.14	68.96	18.91
Immobilized 14d and recovery 14d (USA)					
A350	6.8	197.26	18.85	28.30	52.85

TABLE 2.- BONE PHOSPHORUS CONCENTRATIONS IN RHESUS MONKEY JAWS. EFFECTS OF POSTCRANIAL IMMOBILIZATION

Rhesus number	Body weight (kg)	Total phosphorus (mg/g bone)	Density gradient fractions (sp. gr.) (% of total phosphorus)		
			1.3-1.9	2.0-2.1	2.2-2.9
Control (USA)					
A220	8.2	87.88	27.51	12.63	59.86
A212	6.5	86.25	14.47	8.39	77.14
Immobilized 14d (USA)					
A396	7.7	82.76	13.42	59.52	27.06
72B	7.7	85.07	10.69	19.16	70.15
A392	--	82.35	15.58	22.58	61.84
Immobilized 6° head-down 11d (USSR)					
SRh1	9.5	86.59	12.47	13.24	74.29
SRh2	5.1	84.47	12.70	66.20	21.10
SRh3	5.1	86.99	12.84	68.82	18.33
Immobilized 14d and recovery 14d (USA)					
A350	6.8	84.4	17.88	27.66	54.46

TABLE 3.- BONE HYDROXYPROLINE CONCENTRATIONS IN RHESUS MONKEY JAWS. EFFECTS OF POSTCRANIAL IMMOBILIZATION

Rhesus number	Body weight (kg)	Total OH-Pr	Density gradient fractions (sp. gr.) (% of total OH-Pr)		
			1.3-1.9	2.0-2.1	2.2-2.9
Control (USA)					
A220	8.2	28.94	27.85	13.24	58.91
A212	6.5	28.44	14.37	9.27	76.36
Immobilized 14d (USA)					
A396	7.7	29.15	10.78	61.96	27.25
72B	7.7	29.77	10.23	20.00	69.17
A392	--	29.44	14.45	21.92	63.62
Immobilized 6° head-down 11d (USSR)					
SRh1	9.1	28.53	11.93	14.98	73.09
SRh2	5.1	30.07	12.85	66.06	21.09
SRh3	5.1	32.65	11.82	69.80	18.38
Immobilized 14d and recovery 14d (USA)					
A350	6.8	29.46	17.26	28.20	54.54